



6th IUFRO Meeting Working Party 7-02-09
Phytophthora in Forest and Natural Ecosystems
Meeting Abstracts



Diputación
de Córdoba

CÓRDOBA, SPAIN

9th-14th SEPTEMBER 2012



GOBIERNO
DE ESPAÑA

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

The Sixth Meeting of the International Union of Forest Research Organizations

IUFRO Working Party 7-02-09

Phytophthora in Forests and Natural Ecosystems

Córdoba (Spain)
9th - 14th September
2012



COMMITTEE MEMBERS

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Ajuntament d'Alcoi (Alicante)



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COLLABORATORS



Universidade do Algarve



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6th IUFRO Working Party 7.02.09
“Phytophthora in Forests and Natural Ecosystems”
9th-14th September 2012
Córdoba-Spain

Dear Colleagues,

After the Fifth Meeting of the International Union of Forest Research Organizations IUFRO Working Party 7-02-09 "*Phytophthora* in Forests and Natural Ecosystems" in Rotorua in New Zealand in 2010, we are pleased to hold the Sixth Meeting in Córdoba (Spain). The meeting has been organized jointly by the Universidad de Córdoba and Universitat Politècnica de València.

The city of Córdoba has over two thousand years of history. It was founded in the II century BC by the Roman Consul Claudius Marcellus, keeping the same Iberian name "*Corduba*" (hill near the river) of the former Iberian (Turdetan) village emerged in the late second millenium BC. Córdoba is a living witness and a faithful reflection of powerful civilisations that have settled there. Romans, Moors, Jews and Christians have all left a deep mark on its heritage, culture, traditions and customs. The Mosque was declared a World Heritage in 1984, being extended to the whole old city in 1994.

Furthermore, Córdoba has landscapes derived from Mediterranean forest ecosystems called "dehesas", consisting of pastureland featuring herbaceous species for grazing and tree species belonging to the genus *Quercus*, such as holm oak (*Quercus ilex*) and cork oak (*Q. suber*), although other tree species such pines, wild olives or chestnuts are also present. Two field trips to visit "dehesas" that show different degrees of decline caused by *Phytophthora* have been organized for this meeting.

As previous meetings, we hope that this meeting will be a new occasion to exchange scientific ideas and encourage new collaborations.

Yours sincerely,

The organizing committee



PROGRAMME

6th International Meeting IUFRO Working Party 7.02.09 '*Phytophthora* in Forests and Natural Ecosystems'

SUNDAY, 9 th September 2012	
17:00-20:30	Registration and Poster Set Up
20:30	Departure to Welcome Reception
21:00	Welcome Reception at Palacio de la Merced
MONDAY, 10 th September 2012	
08:30 – 13:00	MORNING FIELD TRIP: Holm oak (<i>Quercus ilex</i>) dehesa
13:30-15:00	LUNCH
15:00-15:30	Welcoming Address: Organising Committee and Local Authorities
15:30-17:30	SESSION 1: EVOLUTION, POPULATION BIOLOGY, EXPERIMENTAL TAXONOMY <i>Chairperson: Everett Hansen</i>
15:30-15:40	Determining the origin of the emerging pathogen, <i>Phytophthora multivora</i> A. REA, G. HARDY, M. STUKELY, T. BURGESS
15:40-15:55	Four phenotypically distinct lineages identified within <i>Phytophthora lateralis</i> C.M. BRASIER, S. FRANCESCHINI, A. M. VETTRAINO, E. M.HANSEN, S. GREEN, C. ROBIN, J. WEBBER, A. VANNINI
15:55-16:10	Population dynamics of aerial and terrestrial populations of <i>Phytophthora ramorum</i> in a California watershed under different climatic conditions M. GARBELOTTO, M. KOZANITAS, C. EYRE
16:10-16:25	Phenotypic diversification is associated with host-induced transposon derepression and repression of crinkler genes in the Sudden Oak Death pathogen M. GARBELOTTO, D.M. RIZZO, T. KASUGA
16:25-16:40	EU2, a fourth evolutionary lineage of <i>Phytophthora ramorum</i> K. VAN POUCKE, S. FRANCESCHINI, J.F. WEBBER, K. HEUNGENS, C.M. BRASIER



16:40- 17:05	Characterization of <i>Phytophthora</i> hybrids from ITS clade 6 associated with riparian ecosystems in South Africa and Australia <u>T. BURGESS</u> , J. NAGEL, D. HÜBERLI, G. HARDY, M. STUKELY, M. WINGFIELD
	Promiscuity, fertility and survival of ITS clade 6 hybrids associated with riparian ecosystems in Western Australia <u>T. BURGESS</u> , D. HÜBERLI, D. WHITE, M. STUKELY, G. HARDY
17:05-17:15	Analysis of the global population structure of <i>Phytophthora plurivora</i> using newly developed, polymorphic SSR markers <u>C. N. SCHOEDEL</u> , S. PROSPERO
17:15-17:20 (+ poster 76)	AFLP analysis reveals low genetic diversity of <i>Phytophthora austrocedrae</i> in Patagonia, Argentina <u>M. L. VÉLEZ</u> , M. P. A. COETZEE, M. J. WINGFIELD, M. RAJCHENBERG, A. G. GRESLEBIN
17:30 – 18:00 REFRESHMENTS	
18:00 – 20:00 DISCUSSION SESSION: ADAPTATION AND EVOLUTION <i>Keynote speakers:</i> "Morphological and physiological adaptability of the genus <i>Phytophthora</i> " <i>Thomas Jung</i> "Fitness, selection and evolutionary divergence in <i>Phytophthora</i> " <i>Clive Brasier</i> "What is a <i>Phytophthora</i> species?" <i>Everett Hansen</i>	
Dinner (self organized) in Córdoba	
TUESDAY, 11th September 2012	
09:00-11:00 SESSION 2: SURVEYS, DIVERSITY, INVASION AND SPREAD <i>Chairperson: Cecile Robin</i>	
09:00-09:15	Role of <i>Phytophthora pseudosyringae</i> in widespread dieback of <i>Nothofagus</i> plantations in Britain <u>B. SCANU</u> , J.F. WEBBER
09:15-09:25	More new <i>Phytophthora</i> species from natural ecosystems in Western Australia A. SIMAMORA, G. HARDY, M. STUKELY, <u>T. BURGESS</u>



09:25-09:40	Landscape-scale epidemic of <i>Phytophthora ramorum</i> on larch in the UK <u>J. F. WEBBER</u> , C. M. BRASIER
09:40-09:55	Evaluating the effect of <i>Phytophthora</i> spp. on tree health in exotic plantations in New Zealand M. DICK, <u>N. WILLIAMS</u> , I. HOOD, J. GARDNER
09:55-10:05	Surveys of soil and water from asymptomatic natural ecosystems in South Africa reveal a goldmine of <i>Phytophthora</i> diversity E. OH, M. WINGFIELD, M. GRYZENHOUT, B. WINGFIELD, <u>T. BURGESS</u>
10:05-10:15	Species of <i>Phytophthora</i> associated with <i>Quercus</i> decline in the Mediterranean Park 'Carrascar de la Font Roja' (Spain) <u>A. PÉREZ-SIERRA</u> , C. LÓPEZ-GARCÍA, M. LEÓN, J. GARCÍA-JIMÉNEZ, P. ABAD-CAMPOS, T. JUNG
10:15-10:25	First approach into the knowledge of the <i>Phytophthora</i> species diversity in Mediterranean Holm oak forests based on 454 parallel amplicon pyrosequencing of soil samples <u>S. CATALÀ</u> , A. PÉREZ-SIERRA, M. BERBEGAL, P. ABAD-CAMPOS
10:25-10:35	Introduction of <i>Phytophthora ramorum</i> in Fagaceae forests in Italy A. VETTRAINO, A. TOMASSINI, N. BRUNI, S. FRANCESCHINI, <u>A. VANNINI</u>
11:00-11:30	COFFEE BREAK
11:30-13:30	SESSION 3: ECOLOGY, EPIDEMIOLOGY AND CLIMATE CHANGE <i>Chairperson: Joan Webber</i>
11:30-11:45	Ectomycorrhizae and <i>Phytophthora cinnamomi</i> relations within the rizosphere of <i>Quercus ilex</i> <u>T. CORCOBADO</u> , A. PÉREZ, M. VIVAS, G. MORENO, A. SOLLA
11:45-11:55	Wildfire influences forest disease dynamics through selective host mortality and pathogen suppression: Sudden oak death in Big Sur, California M. R. METZ, M. M. BEH, K. FRANGIOSO, R. K. MEENTEMEYER, <u>D. M. RIZZO</u>
11:55-12:05	Susceptibility to <i>Phytophthora cinnamomi</i> of the main crops in dehesas and their influence in the epidemiology of the oak root disease <u>M. S. SERRANO</u> , P. DE VITA, P. FERNÁNDEZ, M. E. SÁNCHEZ
12:05-12:15	Sudden oak death impacts to communities and ecosystems in California forests <u>R. C. COBB</u> , D. M. RIZZO



12:15-12:25	Could climate warming be one of the causes of <i>Phytophthora alni</i> emergence in Europe? J. AGUAYO, C. HUSSON, <u>B. MARÇAIS</u>
12:25-12:30 (+ poster 15)	Influence of bird faeces in the behaviour of the root rot of <i>Quercus suber</i> caused by <i>Phytophthora cinnamomi</i> at Doñana Biological Reserve (SW Spain) <u>P. DE VITA</u> , M. S. SERRANO, L. V. GARCÍA, C. RAMO, M. E. SÁNCHEZ
12:30-12:35 (+ poster 23)	Spatial patterns of <i>Phytophthora cinnamomi</i> in declining Mediterranean forests: implications for tree species regeneration <u>L. GÓMEZ-APARICIO</u> , B. IBÁÑEZ, M. S. SERRANO, P. DE VITA, J. M. ÁVILA, I. M. PÉREZ-RAMOS, L. V. GARCÍA, M. E. SÁNCHEZ, T. MARAÑÓN
12:35-12:40 (+ poster 49)	Roads and streams are not significant pathways for SOD spread in tanoak forests E. PETERSON, J. HULBERT, <u>E. M. HANSEN</u>
12:40-12:45 (+ poster 5)	Nature of <i>Phytophthora</i> inoculum in flowing surface waters <u>K. ARAM</u> , D.M. RIZZO
12:45-12:50 (+ poster 32)	Seasonal variation of inoculum density and species composition of soilborne <i>Phytophthoras</i> in an infected black walnut stand in Hungary <u>J. KOVÁCS</u> , F. LAKATOS, I. SZABÓ
12:50-12:55 (+ poster 73)	Epidemiology of <i>Phytophthora ramorum</i> and <i>Phytophthora kernoviae</i> on <i>Vaccinium</i> in the natural environment in the UK <u>G. L. THORP</u> , J. A. TURNER, P. JENNINGS
13:30-15:00	LUNCH
15:00-17:00	SESSION 4: RESISTANCE, PATHOGENESIS, ECOPHYSIOLOGY <i>Chairpersons: Frank Fleischmann / Marilia Horta</i>
15:00-15:15	Blocking of α-plurivirin compromises <i>Phytophthora plurivora</i> pathogenicity towards <i>Fagus sylvatica</i> seedlings <u>R. J. D. DALIO</u> , F. FLEISCHMANN, A. CHAMBERY, R. EICHMANN, N. MASSOLA, S. F. PASCHOLATI, W. OSSWALD
15:15-15:40	Identification of <i>Phytophthora cinnamomi</i> gene transcripts in infected cork oak roots <u>M. HORTA</u> , A. C. COELHO, A. CRAVADOR
	Transcriptome analysis of <i>Quercus suber</i> roots in response to <i>Phytophthora cinnamomi</i> M. HORTA, A. CRAVADOR, <u>A. C. COELHO</u>



15:45-15:55	Quantitative trait loci for resistance to <i>Phytophthora cinnamomi</i> in two host species <u>C. ROBIN</u> , J.M. GION, T. BARRENECHE
15:55-16:05	Interactions between <i>Phytophthora cinnamomi</i> and <i>Phlomis purpurea</i>, a plant resistant to the pathogen <u>D. NEVES</u> , C. MAIA, S. DURAES, M. HORTA, S. CAMPOS, M. C. MATEUS, F. GARCÍA BREIJO, J. R. ARMINANA, A. CRAVADOR
16:05-16:15	How do <i>Phytophthora</i> spp. harm woody plants?. W. ORWALD, <u>F. FLEISCHMANN</u> , T. CECHE, A. CHAMBERY, A. CRAVADOR, S. DIAMANDIS, J. DÍEZ CASERO, A.C. COELHO, E. HANSEN, M. HORTA, T. JUNG, N. S. MASSOLA, L.B. ORLIKOWSKI, S. PROSPERO, M. PTASZEK, D. RIGLING, C. ROBIN, A. SOLLA, G. SIPOS, A. TRZEWIK, S. WERRES
16:15-16:25	Multitrophic interactions between <i>Quercus robur</i>, <i>Phytophthora quercina</i> and <i>Piloderma croceum</i> (on a joint experimental platform) <u>O. ANGAY</u> , F. FLEISCHMANN, S. RECHT, S. HERMANN, T. GRAMS
16:25-16:35	Drought, fire and flood: approaches to the renewed challenge of <i>Phytophthora cinnamomi</i> in south-eastern Australia <u>D. CAHILL</u> , J. ROOKES, J. CULLUM, H. BRAMWELLS, D. PHILLIPS, P. BEECH
17:00-18:30	POSTER SESSION I + REFRESHMENTS
	Dinner (self organised) in Córdoba
21:00	Evening visit to the old city
WEDNESDAY, 12th September 2012	
08:00-20:00	FIELD TRIP TO ANDALUSIAN MEDITERRANEAN FORESTS: Holm oak - <i>P. cinnamomi</i> and Wild olive - <i>P. megasperma</i> and <i>P. inundata</i> Can limestone amendments and pig slurry be considered as control methods for <i>Quercus ilex</i> root rot caused by <i>Phytophthora cinnamomi</i> in dehesa? Preliminary results from field trials M. S. SERRANO, J. R. LEAL, , P. DE VITA, P. RÍOS, P. FERNÁNDEZ, M. E. SÁNCHEZ Holm oak regeneration in dehesas with presence of the soilborne pathogen <i>Phytophthora cinnamomi</i>: something to do? M. S. SERRANO, P. DE VITA, A. M. GARCÍA, M. D. CARBONERO, J. R. LEAL, M. E. SÁNCHEZ, P. FERNÁNDEZ



	<i>Phytophthora</i> root rot in a wild olive forest (<i>P. megasperma</i> and <i>P. inundata</i>) M. E. SÁNCHEZ, A. TRAPERO, C. M. BRASIER
	Dinner (self organised) in Córdoba
THURSDAY, 13th September 2012	
10:00-11:00	SESSION 5: SURVEYS, DIVERSITY, INVASION AND SPREAD (cont. Session 2) / CLASSICAL TAXONOMY AND CLASSICAL MOLECULAR DIAGNOSTICS <i>Chairperson: Ellen Goheen</i>
10:00-10:10	<i>Phytophthora</i> species associated with <i>Alnus rubra</i> in western Oregon riparian ecosystems <u>L. SIMS</u> , E. HANSEN, S. NAVARRO
10:10-10:15 (+ poster 51)	<i>Phytophthora ramorum</i> and <i>P. lateralis</i> in Northern Ireland L. QUINN, <u>A. MCCracken</u> , B. MORELAND
10:15-10:20 (+ poster 4)	<i>Phytophthora bilorbang</i> prov. nom., a new species associated with declining <i>Rubus anglocandicans</i> (blackberry) in Western Australia <u>S. AGHIGHI</u> , G. HARDY, J. K. SCOTT, T. BURGESS
10:20-10:25 (+ poster 12)	Spatial patterns of holm and cork oak decline in Extremadura, Spain <u>E. CARDILLO</u> , A. ACEDO, C. PEREZ
10:25-10:30 (+ poster 40)	<i>Phytophthora</i> species in Serbia <u>I. MILENKOVIC</u> , N. KECA, J. NOWAKOWSKA, K. SIKORA, M. BORYS, T. OSZAKO, T. JUNG
10:30-10:35 (+ poster 9)	A species concept for <i>Phytophthora</i> taxon <i>Agathis</i> (PTA) — causal agent of root and collar rot of <i>Agathis australis</i> in New Zealand <u>S. E. BELLGARD</u> , B. S. WEIRA, P. R. JOHNSTON, D. PARK, D. J. THAN, N. ANAND, S. R. PENNYCOOK
10:35-10:40 (+ poster 54)	<i>Phytophthora</i> ITS Clade 3 expands to include a sixth new species, <i>P. taxon pluvialis</i> <u>P. W. REESER</u> , W. SUTTON, E.M. HANSEN
10:40-10:45 (+ poster 21)	<i>Phytophthora acerina</i> prov. nom., a new species from the <i>P. citricola</i> complex causing aerial cankers on <i>Acer pseudoplatanus</i> in Italy <u>B. GINETTI</u> , S. MORICCA, A. RAGAZZI, T. JUNG
11:00-11:30	COFFEE BREAK



11:30-12:00	SESSION 6: RESISTANCE, PATHOGENESIS, ECOPHYSIOLOGY (cont. Session 4) Chairperson: Ana Pérez Sierra
11:30-11:35 (+ poster 38)	A new method to quantify zoospore chemo-attraction N.S. MASSOLA JR., R. DALIO, <u>F. FLEISCHMANN</u> , W. OSSWALD
11:35-11:40 (+ poster 33)	Fluorescent <i>in situ</i> hybridization (FISH) assay as a tool to microscopically view <i>Phytophthora cinnamomi</i> growth within plant tissues A. LI, M. CRONE, P. ADAMS, S. FENWICK, N. WILLIAMS, <u>G. HARDY</u>
11:40-11:45 (+ poster 52)	Histological changes of <i>Quercus ilex</i> seedlings infected by <i>Phytophthora cinnamomi</i> M.A. REDONDO, A. PÉREZ-SIERRA, <u>P. ABAD-CAMPOS</u> , J. GARCÍA-JIMÉNEZ, A. SOLLA, J. REIG, F. GARCÍA-BREIJO
11:45-11:50 (+ poster 14)	Quick dissemination of <i>Phytophthora cinnamomi</i> threatens biodiversity in a World Heritage Site (Doñana Biological Reserve, SW Spain) P. DE VITA, L. V. GARCÍA, C. RAMO, M. S. SERRANO, C. APONTE, <u>M. E. SÁNCHEZ</u>
12:00-13:30	SESSION 7: MANAGEMENT AND CONTROL Chairperson: Matteo Garbelotto
12:00-12:20	A chronicle of the impacts of sudden oak death in California: Fifteen years of invasion <u>S. J. FRANKEL</u>
12:20-12:40	Monitoring the effectiveness of <i>Phytophthora ramorum</i> eradication treatments in Oregon Tanoak Forests <u>E. M. GOHEEN</u> , A. KANASKIE, E. HANSEN, W. SUTTON, P. REESER, N. OSTERBAUER
	Managing sudden oak death in Oregon forests, 2001-2011 <u>A. KANASKIE</u> , M. MCWILLIAMS, S. SAVONA, E. HANSEN, W. SUTTON, P. REESER, E. M. GOHEEN, D. HILBURN, N. OSTERBAUER
12:40-12:50	Mitigating against dispersal of <i>Phytophthora</i> spp. on forest produce <u>I. HOOD</u> , M. DICK, N. ARHIPOVA, J. GARDNER, P. TAYLOR
12:50-13:00	Survival and eradication of <i>Phytophthora cinnamomi</i> from black gravel graveyard sites in the <i>Eucalyptus marginata</i> (jarrah) forest M. CRONE, J. MCCOMB, P. O'BRIEN, V. STOKES, I. COLQUHOUN, <u>G. HARDY</u>
13:00-13:05 (+ poster 80)	Investigations on control measures for <i>Phytophthora ramorum</i> and <i>Phytophthora kernoviae</i> in heritage gardens and parks <u>E. F. WEDGWOOD</u> , D. LOCKLEY, J. TURNER, G. THORP, B. HENRICOT



13:30-15:00	LUNCH
15:00 – 17:00	<p>DISCUSSION SESSION: THE NURSERY PATHWAY Keynote speakers:</p> <p>"Ubiquitous <i>Phytophthora</i> infestations of nurseries and plantings in Europe demonstrate major failure of plant biosecurity" <i>Thomas Jung</i></p> <p>"Patterns of repeated introductions and migrations of <i>P. ramorum</i> in North America" <i>Nik Grünwald</i></p>
17:00 – 18:30	POSTER SESSION II + REFRESHMENTS
18:30-19:30	<p>DISCUSSION SESSION: GEOGRAPHIC ORIGINS OF SPECIES Keynote speakers:</p> <p>"Searching for <i>Phytophthoras</i> in the forests of Nepal and Taiwan" <i>Andrea Vannini</i></p> <p>"Variation in <i>Phytophthora</i>: a key to historical pathways?" <i>Frans Arentz</i></p> <p>"Are tropical forests <i>Phytophthora</i> hot spots?" <i>Yilmaz Balci</i></p>
20:30	LEAVE FOR CONFERENCE DINNER (Bodegas Campos)
FRIDAY, 14th September 2012	
09:15 – 09:45	Announcements
09:15-09:25	<p>Forest <i>Phytophthoras</i>, a new international journal and website <u>J. PARKE</u>, J. EBERHART</p>
09:25-09:35	<p>Morphological-Molecular ID Tools of <i>Phytophthora</i>: Lucid & Tabular Keys and Sequencing Analysis <u>Z. G. ABAD</u>, Y. BALCI, M. COFFEY, S. KANG</p>



09:45-10:45	Session 8: MANAGEMENT AND CONTROL IN AUSTRALIA <i>Chairperson: Susan Frankel</i>
09:45-10:15	The state of <i>Phytophthora</i> science and management in natural ecosystems is Australasia <u>G. HARDY, N. WILLIAMS</u>
	A comparison between liquid phosphite injections and novel soluble phosphite and nutrient implants to control <i>Phytophthora cinnamomi</i> in <i>Banksia grandis</i> and <i>Eucalyptus marginata</i> P. SCOTT, P. BARBER, <u>G. HARDY</u>
10:15-10:25	Community involvement in <i>Phytophthora</i> dieback management - looking back and forward <u>J. COLQUHOUN</u> , C. DUNNE, J. YOUNG
10:25-10:35	Successful containment and eradication of <i>Phytophthora cinnamomi</i> at a management level from diverse natural ecosystems in Western Australia C. DUNNE, R. HARTLEY, W. DUNSTAN, P. SCOTT, T. PAAP, <u>G. HARDY</u>
10:45-11:15	COFFEE BREAK
11:15-12:15	General Discussion. Meeting Summary: <i>Andrea Vannini and Joan Webber</i>
12:15-13:00	Wrap up session: Regional Chairs
13:30-14:30	LUNCH
14:30	DEPARTURE TO VALENCIA



POSTERS	
1	Assessment of the presence of oomycetes in forest nurseries in Eastern Spain. <u>P. ABAD-CAMPOS</u> , A. PUÉRTOLAS, A. ORTEGA, P. GUIRAO, M. LEÓN, J. GARCÍA-JIMÉNEZ, A. PÉREZ-SIERRA
2	Effect of three control treatments on the survival of <i>Quercus ilex</i> seedlings infected with <i>Phytophthora cinnamomi</i> . <u>A. ACEDO</u> , E. CARDILLO, I. NÚÑEZ
3	Pythiaceae and fungal species isolated from coniferous and deciduous seedlings in some Turkish nurseries. <u>A.G. ADAY</u> , A. LEHTIJÄRVI, H.T. DOĞMUŞ-LEHTIJÄRVI
4	<i>Phytophthora bilorbang</i> prov. nom., a new species associated with declining <i>Rubus anglocandicans</i> (blackberry) in Western Australia. <u>S. AGHIGHI</u> , G. HARDY, J. K. SCOTT, T. BURGESS
5	Nature of <i>Phytophthora</i> inoculum in flowing surface waters. <u>K. ARAM</u> , D.M. RIZZO
6	Characterisation of the two informally designated ITS Clade 6 taxa <i>Phytophthora</i> taxon Forestsoil and <i>P. sp. hungarica</i> . <u>J. BAKONYI</u> , Z. Á. NAG, T. BURGESS, A. SZIGETHY, J. NECHWATAL, A. KOLTAY, S. WOODWARD, L. BELBAHRI, T. JUNG
7	Population fluctuation of <i>Phytophthora</i> spp in streams and their possible role as early colonizers. <u>Y. BALCI</u>
8	<i>Phytophthora</i> species associated with disease in peri-urban woodland and forest ecosystems. <u>P. BARBER</u> , T. BURGESS, T. PAAP, G. HARDY
9	A species concept for <i>Phytophthora</i> taxon <i>Agathis</i> (PTA) — causal agent of root and collar rot of <i>Agathis australis</i> in New Zealand. <u>S. E. BELLGARD</u> , B. S. WEIRA, P. R. JOHNSTON, D. PARK, D. J. THAN, N. ANAND, S. R. PENNYCOOK
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70	Calcium supplementation of soil augments the control of <i>Phytophthora cinnamomi</i> by phosphite. P. STASIKOWSKI, J. MCCOMB, B. SHEARER, P. O'BRIEN, G. HARDY
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80	Investigations on control measures for <i>Phytophthora ramorum</i> and <i>Phytophthora kernoviae</i> in heritage gardens and parks. E. F. WEDGWOOD, D. LOCKLEY, J. TURNER, G. THORP, B. HENRICOT
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83	Characterising the distribution of <i>Phytophthora</i> taxon Agathis (PTA) in the bark, cambium, and wood of diseased New Zealand kauri (<i>Agathis australis</i>). M.P. WHEAT, S.E. BELLGARD
84	<i>Phytophthora cinnamomi</i> infection results in changed patterns of fine root production in Scots pine. ELENÍ SIASOU, HELENA BRAGANCA AND STEVE WOODWARD
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87	New hypothesis on the ploidy of the hybrid species <i>Phytophthora alni</i> subsp. <i>alni</i> . C. HUSSON, J. AGUAYO, P. FREY, B. MARÇAIS



SESSION 1

EVOLUTION, POPULATION BIOLOGY, EXPERIMENTAL TAXONOMY



Determining the origin of the emerging pathogen, *Phytophthora multivora*

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Phytophthora multivora is widespread in Western Australia (WA); it has a wide host range and considerably variability in the sequence of the mitochondrial gene *cox1* led to the hypothesis that it may be endemic to the region. To test this hypothesis, four nuclear (ITS, *enlase*, *HSP90* and *ras*) and three mitochondrial (*cox1*, *cox1GS* and *nadh1*) loci were sequenced for 60 isolates of *P. multivora* isolated from Australia, South Africa (RSA) and Europe and the data were subjected to phylogenetic, coalescent-based and population genetic analyses. Isolates from RSA possess greater nucleotide diversity and a greater number of alleles at three of the nuclear loci and at all three mitochondrial loci than those from WA. In addition, the RSA population had more unique multilocus genotypes than the WA population. While *P. multivora* is widely distributed in natural ecosystems in WA and RSA, it is usually isolated from nurseries or horticulture elsewhere in the world. Additionally, *P. multivora* is consistently isolated from cankers and dead and dying plants of numerous endemic hosts in WA, but is predominantly isolated from soil associated with asymptomatic plants in RSA. Based on this evidence it is proposed that *P. multivora* is endemic to RSA and has been introduced to Western Australia.

Four phenotypically distinct lineages identified within *Phytophthora lateralis*

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Until recently *Phytophthora lateralis* was known only as the cause of dieback and mortality of *Chamaecyparis lawsoniana* in its native range in the Pacific Northwest [1]. Since the 1990s however disease outbreaks have occurred increasingly on ornamental *C. lawsoniana* in Europe; and in 2007 the pathogen was discovered in soil around old growth *C. obtusa* in Taiwan, where it may be endemic [2]. When the phenotypes of over 150 isolates of *Phytophthora lateralis* from Taiwan; across the Pacific Northwest (British Columbia to California); and from France, the Netherlands and the UK were compared three well separated growth rate groups were resolved: one from Taiwan, one from the Pacific Northwest and Europe and one from a small area of the UK. Among these groups nine distinct types were identified based on colony patterns and spore metrics and discriminated in a multivariate analysis. The assumption that the three main growth rate groups represented distinct phylogenetic units was tested by comparative sequencing of two mitochondrial and three nuclear genes. This assumption was confirmed. In addition two phenotype clusters within the Taiwan growth group were also shown to be separate lineages. The characteristics and distribution of the four *P. lateralis* lineages will be presented and their evolutionary, taxonomic and plant health significance discussed.

[1] Hansen E.M., Goheen D.J., Jules E.S. and Ullian B. Managing Port-Orford-Cedar and the introduced pathogen *Phytophthora lateralis*. *Plant Disease* **84** 4-14 (2000).

[2] Brasier C.M., Vettraino A.M., Chang T.T. and Vannini A. *Phytophthora lateralis* discovered in an old growth *Chamaecyparis* forest in Taiwan. *Plant Pathology* **59** 595-603 (2010).



Population dynamics of aerial and terrestrial populations of *Phytophthora ramorum* in a California watershed under different climatic conditions

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In the attempt to understand the epidemiology of Sudden Oak Death in California, we present the first combined genetic analysis of *P. ramorum* from soil and leaves. Successful isolations from leaves of the transmissive host California bay laurel increased from 15 to 39% between dry and wet conditions. Symptoms caused by other foliar pathogens were highest (69%) in dry conditions, suggesting that *P. ramorum* and other pathogens are favored by different climatic conditions. Some foliar genotypes of *P. ramorum* were more abundant in wet than in dry conditions and persistent through time. Soil and foliar populations were genetically distinct, but were not segregated in different portions of a minimum spanning network, suggesting intermixing of the two. We surmise that the genetic structure between substrates is not due to the presence of two distinct populations, but to the different ability of genotypes to adapt to different substrates. To support this hypothesis, we show that ranking of genotypes based on abundance are clearly different between soil and leaf populations. We provide evidence that in climatic conditions unfavorable to the pathogen, genetic diversity increases both within and between sites, while in favorable conditions diversity decreases due to greater migration levels of some genotypes. Finally, we show that foliar genotypes can spread further than soil genotypes in wet years, and that soil appears to be re-inoculated on a yearly basis. Cumulatively, results indicate leaves act as a relatively persistent source of inoculum, while soil is potentially inconsequential for the natural spread of the disease.

Phenotypic diversification is associated with host-induced transposon derepression and repression of crinkler genes in the Sudden Oak Death pathogen

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Phytophthora ramorum is responsible for sudden oak death in California. *P. ramorum* is a generalist pathogen with over 100 known host species. Three or four closely related genotypes of *P. ramorum* (from a single lineage) were originally introduced in California forests and the pathogen reproduces clonally. Because of this, the genetic diversity of *P. ramorum* is extremely low in Californian forests. However, *P. ramorum* shows diverse phenotypic variation in colony morphology, colony senescence, and virulence. In this study, we show that phenotypic variation among isolates is associated with the host species from which the microbe was originally cultured. Microarray global mRNA profiling detected derepression of transposable elements (TEs) and down-regulation of crinkler effector homologs (CRNs) in the majority of isolates originating from coast live oak (*Quercus agrifolia*), but this expression pattern was not observed in isolates from California bay laurel (*Umbellularia californica*). In some instances, oak and bay laurel isolates originating from the same geographic location had identical genotypes based on multilocus simple sequence repeat (SSR) marker analysis but had different phenotypes. Expression levels of the two marker genes analyzed by quantitative reverse transcription PCR were correlated with originating host species, but not with multilocus genotypes. Because oak is a non-transmissible dead-end host for *P. ramorum*, our observations are congruent with an epi-transposon hypothesis; i.e., physiological stress is triggered on *P. ramorum* while colonizing oak stems and disrupts epigenetic silencing of TEs. We propose the *P. ramorum*-oak host system in California forests as an ad hoc model for epi-transposon mediated diversification.



EU2, a fourth evolutionary lineage of *Phytophthora ramorum*

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Studies in North America and Europe over the past decade have demonstrated the occurrence of three lineages of *Phytophthora ramorum* informally designated the NA1, NA2 and EU1 lineages. Each lineage appears to represent a reproductively isolated population, but whether they have come from different geographic regions is unknown. Only the EU1 lineage had been found in Europe until recently. EU1 is believed to have been introduced into Europe around 1990. Since then it has spread widely and rapidly across the continent, including the UK and Ireland, via the plant trade. In 2011 *P. ramorum* isolates from Northern Ireland and a closely adjacent area of western Scotland, mostly from *Larix* but also from *Quercus*, *Rhododendron* and *Vaccinium*, were found to have molecular profiles not matching those of any known lineage. Following a phylogenetic study based on eleven polymorphic loci and an SSR analysis they were assigned to a new lineage, informally designated EU2. This analysis indicates the EU2 lineage may be ancestral to the other lineages. No SSR-based intra-EU2 lineage genotypic diversity was detected. All EU2 isolates examined to date have all been of A1 mating type. As this is the same mating type as that of EU1 in Europe, sexual recombination with EU1 lineage genotypes already resident in the UK is unlikely. The earliest isolation dates to 2007. Present evidence points to a recent introduction of EU2 in the context of ongoing phytosanitary emergency measures. The arrival of EU2 highlights an urgent need to identify the geographic origins of *P. ramorum* in order to understand the organism's natural ecology, the processes that have produced the lineages, and whether further lineages exist. Presently, studying the organism in the context of introduction and invasion, we may only be looking at half the picture.

Characterization of *Phytophthora* hybrids from ITS clade 6 associated with riparian ecosystems in South Africa and Australia

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Recent surveys of Australian and South African rivers have revealed numerous Clade 6 *Phytophthoras*, which either have ITS gene regions that were highly polymorphic or could not be sequenced. These isolates were suspected to be hybrids. In order to establish the hybrid nature of these isolates, three nuclear loci and one mitochondrial locus were amplified and, in the case of the nuclear gene regions cloned, and sequenced. Abundant recombination within the ITS region was observed and this combined with phylogenetic comparison of other three loci confirmed the presence of four distinct hybrids involving three known parental species: *P. amnicola*, *P. thermophila* and *P. taxon* PgChlamydo. In each case the hybrid is between two parental species. For the single copy nuclear genes (ASF and GPA) examined, two alleles were obtained, one of which corresponded to each of the parental species. In all cases, only a single *cox1* allele was obtained indicating that mitochondria were always uniparentally inherited from one of the nuclear parents. This pattern of nuclear and mitochondrial inheritance suggests that each hybrid is a result of an independent hybridization event involving two parental species. The hybrid species are sterile and have physiological traits similar to those of the maternal parental. The pathogenicity of these hybrids is unknown, but several isolates from Western Australia were obtained from the rhizosphere soil of dying plants. The serendipitous and simultaneous discovery of the same hybrid complex on two continents is intriguing. However, the wide geographic distribution,



frequent isolation and presence of all four hybrids and all parental species suggest that their origin lies in Australia. The association of the sampled riverways with botanical gardens in South Africa containing Australian plants may be a clue to the pathway of introduction.

Promiscuity, fertility and survival of ITS clade 6 hybrids associated with riparian ecosystems in Western Australia

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Over the past few years several large scale *Phytophthora* surveys have been undertaken in Western Australia. In all cases, the ITS region of numerous isolates obtained from water or riparian soil have been unsequencable. These isolates are hybrids, all involving parental species from ITS clade 6, sub-clade II. Parental species are *P. thermophila* (T), *P. fluvialis* (F), *P. litoralis* (L), *P. amnicola* (A) and *P. taxon stagnum* (S). In most cases, two alleles were found for the nuclear genes and a single allele for the mitochondria gene, suggesting that each hybrid is a result of an independent sexual hybridization event involving two parental species with the mitochondria inherited from the maternal parent. To date the following hybrids have been characterised (maternal parent first); A-F, A-S, F-S, L-S, T-A, T-S and S-F. The hybrid isolates all appear to be sterile and readily produce sporangia on soil extract, however, some of them produced unusual or aborted sporangia. The growth and colony pattern produced by the hybrids on three different agar media is similar to that of the maternal parent. These hybrids have predominantly been isolated from natural waterways but some have been isolated from the rhizosphere soil of dying plants. There is no evidence of subsequent hybridization events (back crossing or hybrids crossing with hybrids), but this cannot be ruled out at this stage. The two parental ITS alleles are combined and subsequently recombined through mitotic recombination events creating significant variation between the rDNA subunits. The fact that this occurs suggests that the hybrids are relatively stable and are able to survive without resting structures, probably through continual sporulation within riparian ecosystems. Their role in the environment remains a mystery.

Analysis of the global population structure of *Phytophthora plurivora* using newly developed, polymorphic SSR markers

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Phytophthora species are well known to cause devastating diseases on numerous crops, ornamentals, and native plants. In recent years, their spread has been accelerated by the increasing international trade of woody plants. However, with a few exceptions (e.g. *P. ramorum*, the causal agent of Sudden Oak Death), detailed knowledge on the global population structure and the pathways of spread of forest *Phytophthora* species is still missing. This lack of knowledge is mainly due to the absence of appropriate species-specific molecular markers.

P. plurivora, a member of the *P. citricola* species complex, is involved in widespread beech (*Fagus sylvatica*) and oak (*Quercus* sp.) declines in natural and semi-natural forest ecosystems in Europe (1). Moreover, this species is frequently found in European ornamental nurseries and has been reported in North American nurseries and plantations not long ago.

We recently developed polymorphic SSR markers for *P. plurivora* (2) and used them to analyze the



worldwide population structure, genotyping ~500 isolates from 18 countries of this cosmopolitan plant pathogen. Here, we will present the results of this study which aimed (a) to determine whether Europe can be considered as the center of origin of *P. plurivora*, and (b) to characterize the pathways of spread of *P. plurivora* within Europe and between Europe and US.

[1] Jung, T. and Burgess, T. I.. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia*, **22** 95–110 (2009).

[2] Schoebel, C. N. and Prospero, S. Development of new, polymorphic SSR markers for three closely related plant pathogenic *Phytophthora* species using 454 next generation sequencing (in preparation) (2012).

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AFLP analysis reveals low genetic diversity of *Phytophthora austrocedrae* in Patagonia, Argentina

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Phytophthora austrocedrae is a recently discovered pathogen that causes high levels of mortality of *Austrocedrus chilensis* in Patagonia. The susceptibility of the host tree together with the dispersal pattern of the pathogen in Patagonia led to the hypothesis that *P. austrocedrae* was introduced into Argentina. But the genetic diversity of the pathogen that would better inform this question has not been determined. The aim of this study was, therefore, to assess the population structure of *P. austrocedrae* isolates from Argentina in order to gain an understanding of the origin and spread of the pathogen. Genetic diversity was determined based on amplified fragment length polymorphisms (AFLPs). In total, 48 isolates of *P. austrocedrae* were obtained from infected *A. chilensis* trees, and these represented the geographical range of the host. Four primer combinations were used for the AFLP analysis. Of the 332 scored bands, 12% were polymorphic. Gene diversity (*h*) ranged from 0.0050-0.0258 and the Shannon index (*I*) from 0.0180-0.0432. A high degree of genetic similarity was observed among the isolates (pairwise *S* values= 958-1; 0.993 ± 0.009 , mean \pm S.D). A frequency histogram showed that most of the isolate pairs were 100% similar. Principal coordinate analysis using three-dimensional plots did not group any of the isolates based on their geographical origin. The low genetic diversity (within and between sites) and absence of population structure linked to geographic origin, suggest that *P. austrocedrae* was introduced into Argentina.



SESSION 2

SURVEYS, DIVERSITY, INVASION AND SPREAD



Role of *Phytophthora pseudosyringae* in widespread dieback of *Nothofagus* plantations in Britain

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Since 2009 extensive dieback and mortality of *Nothofagus obliqua* associated with bleeding cankers on stem and branches has been observed in Britain. The casual agent of this disease was identified as *Phytophthora pseudosyringae*, based on morphological and ITS analysis. In 2011 a survey was undertaken to assess the frequency and type of *P. pseudosyringae* infections, the comparative susceptibility of *N. obliqua* and other different woody hosts, and the sporulation potential of *P. pseudosyringae* on *Nothofagus* foliage. Infections of *P. pseudosyringae* on *Nothofagus* appeared to be widespread in Britain with infected trees being found on at least three sites in England, two in Scotland and one in Wales. Additional symptoms such as twig blight and leaf necrosis suggested that aerial infection was occurring. Besides *N. obliqua*, also *Nothofagus alpina*, *Fagus sylvatica* and *Vaccinium myrtillus* were found to be infected. In pathogenicity tests *P. pseudosyringae* was shown to be an effective bark pathogen of *Nothofagus*, but with significant differences between the different woody hosts assayed. Susceptibility of foliage showed marked differences between the host species tested, with *N. obliqua* leaves proving to be highly susceptible. The high levels of sporulation observed on infected *N. obliqua* leaves suggests that *P. pseudosyringae* has the potential to sporulate heavily on foliage and spread from there to shoots, branches and stems. *P. pseudosyringae* on *Nothofagus* represents the third *Phytophthora* species causing aerial infection on forest trees in the UK and could have the potential to pose a serious threat to *Nothofagus* in its native southern hemisphere.

More new *Phytophthora* species from natural ecosystems in Western Australia

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In 2009 (Plant Dis. 93:215-223) we reported 11 apparently new *Phytophthora* species, designated P.sp.1-11, from natural ecosystems in Western Australia (WA). Since then many of these species have been described: *P. multivora* (P.sp.4), *P. elongata* (P.sp.2), *P. constricta* (P.sp.9), *P. arenaria* (P.sp.1), *P. thermophila* (P.sp.3), *P. litoralis* (P.sp.11), *P. gregata* (P.sp.7), *P. fluvialis* (P.sp.8). P.sp.5 falls in the *P. cryptogea* species complex and P.sp.6 has been identified as *P. taxon personii*. Additionally we have described *P. gibbosa* and *P. amnicola*. Further sampling and continued molecular re-evaluation of the culture collection at the Department of Environment and Conservation's Vegetation Health Service (VHS) has uncovered more new species tentatively named *P. aff. humicola*, *P. aff. rosacearum*, *P. aff. elongata*, *P. aff. arenaria*, *P. aff. captiosa*, *P. taxon. kwongan* (=P.sp.10), *P. taxon. casuarina*. A large number of the new species are from ITS clade 6, sub-clade I and they have been isolated in remote natural vegetation. All known species and taxa in sub-clade I, with the exception of *P. humicola*, have been isolated in WA, perhaps illuminating a WA origin for this clade. Studies are currently underway to formally describe the new species in conjunction with large scale pathogenicity trials of these and the other newly described species.



Landscape-scale epidemic of *Phytophthora ramorum* on larch in the UK

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Phytophthora ramorum has been reported from most EU Member States, mainly affecting ornamental plants in nurseries. The most epidemiologically important hosts are those that support abundant sporulation and until recently in Europe this applied primarily to rhododendron and *Vaccinium*. However, following the first findings of *P. ramorum* on Japanese larch (*Larix kaempferi*) in south west Britain in 2009, it soon became clear that infected needles of *Larix* species, especially *L. kaempferi*, (Japanese larch) could produce abundant numbers of sporangia, as demonstrated both in the laboratory and on naturally infected trees. Moreover, as larch is also a canker host, the infected foliage then causes stem infections on larch as well as on other nearby susceptible trees including broadleaf and conifer species.

Since 2009 it has become clear that *ramorum* disease of larch is now throughout the western side of the UK where the climate is especially conducive to infection and sporulation. To control disease spread millions of trees have now been felled and aerial surveillance is a vital part of disease detection. All three larch species that are grown commercially in the UK (*L. kaempferi*, *L. decidua* and *L. x eurolepis*) can become naturally infected although they differ in their bark and foliar susceptibility. The current epidemic raises many challenges to our understanding of the disease including how *P. ramorum* may be changing in this new environment, the need for improved diagnostic methods for conifer material, and whether other *Phytophthora* species may also be associated with larch but have previously gone undetected because of cryptic symptom development.

Evaluating the effect of *Phytophthora* spp. on tree health in exotic plantations in New Zealand

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Historically the exotic forest plantations in New Zealand have been largely free of diseases caused by *Phytophthora* spp. in spite of the presence of some capable pathogens such as *P. cinnamomi* and *P. kernoviae*. However because of the increasing number of *Phytophthora* species causing novel diseases world-wide a re- evaluation of those isolated from New Zealand forestry species and their association with symptoms of disease was undertaken.

In a survey programme covering four years, stands aged from 2-28 years in plantation forests across the North Island were assessed for wilt, dieback, stem and branch cankers and needle disease that could not be attributed to known biotic and abiotic causal agents. Where non-attributable conditions were identified a potential association with an oomycete(s) was sought through isolation, by ELISA test (followed by PCR confirmation), and through microscopic examination of tissues.

Some novel host/*Phytophthora* associations of undetermined significance were encountered and these will be discussed.



Surveys of soil and water from asymptomatic natural ecosystems in South Africa reveal a goldmine of *Phytophthora* diversity

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Phytophthora species are well known as destructive plant pathogens, especially in natural ecosystems. However, little is known regarding the *Phytophthora* diversity in forests of South Africa. In this study, *Phytophthora* species were isolated using standard baiting techniques from 150 soil and water samples and these were identified based on ITS and *cox1* sequence data. The 162 resulting *Phytophthora* isolates resided in 11 taxa including four known species (*P. multivora*, *P. capensis*, *P. frigida*, *P. cinnamomi*) the known but as yet unnamed, *Phytophthora* taxon PgChlamydo and *P. taxon emzanzi* and five new taxa. The most commonly isolated species from soil was *P. multivora* (75%), a species recently described from Western Australia where it has been extensively associated with dead and dying trees. *P. capensis* and *P. taxon emzanzi* have recently been described from the Cape region of South Africa and *P. multivora* was also reported from this region. The extensive isolation of *P. multivora* from asymptomatic natural vegetation suggests that South Africa may be the origin of this species. *P. frigida* was isolated for the first time from stream water and *P. taxon PgChlamydo* was isolated for the first time in Africa. The new species were isolated from water and not surprisingly belong to ITS Clades 6 and 9. With the exception of *P. cinnamomi*, very little is known regarding the biology, epidemiology or origin of these species.

Species of *Phytophthora* associated with *Quercus* decline in the Mediterranean Park 'Carrascar de la Font Roja' (Spain)

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The Natural Park Carrascar de la Font Roja is one of the best preserved nature areas (2298 Ha.) in Comunidad Valenciana (Eastern Spain) that includes the Menejador mountain range, with the highest altitude of 1356 m. The forest is dominated by Mediterranean *Quercus* spp., ie. *Quercus ilex*, *Q. faginea* and *Q. coccifera*. During the last decade a severe decline of oaks has been observed. The symptoms include dieback of branches and parts of the crown, increased transparency of the crown, withering of leaves and death of trees. A significant decrease in the production of acorns and saplings affecting natural regeneration has also been observed. No previous studies on a possible involvement of *Phytophthora* and other oomycetes in the decline have been carried out. Therefore, during 2010-2011 soil samples from affected trees were collected and their roots examined. An extensive loss of both lateral small woody roots and fine roots and callusing or open cankers were observed. Soil samples containing fine roots from declining trees were baited using both *Q. robur* leaves and apple fruits as baits. Six *Phytophthora* species were detected: *P. cryptogea*, *P. gonapodyides*, *P. megasperma*, *P. quercina*, *P. psychrophila* and *P. syringae*. Pathogenicity test with representative isolates of these species were conducted for six months under control conditions with one-year old seedlings of *Q. ilex* and *Q. faginea*. *P. cinnamomi* was included in the pathogenicity test for comparison. The results showed that *Q. ilex* seedlings were more susceptible to infection than *Q. faginea* with *P. cinnamomi* and *P.*



megasperma causing severe reduction in root biomass. The role of all *Phytophthora* species detected in the decline of Mediterranean *Quercus* spp. is discussed.

First approach into the knowledge of the *Phytophthora* species diversity in Mediterranean Holm oak forests based on 454 parallel amplicon pyrosequencing of soil samples

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The evergreen holm oak (*Quercus ilex* L.) is the most representative tree species in the Iberian Peninsula and the main tree in oak-rangeland ecosystems (dehesas). Among the most important problems that threaten the persistence of oak groves and dehesas are the absence of natural regeneration and root rot due to *Phytophthora cinnamomi*. Recently, new species of *Phytophthora* other than *P. cinnamomi* were detected in holm oak forest using traditional baiting methods and isolation. Since it is not easy to detect, identify or quantify *Phytophthora* species, the need arises to find a rigorous technique, which is rapid, reliable and highly reproducible to evaluate their diversity in living in holm oak forests. In this study, the polymorphic ITS1 region is used to evaluate the presence of *Phytophthora* species in soil samples. Tagged amplicons were obtained with *Phytophthora* template-specific primers which excluded other oomycetes and fungi. Similarity of the barcoded reads obtained by 454-pyrosequencing after BLAST against GenBank database and comparison of abundance was assessed from three different soil samples: from trees showing decline symptoms, rainfall runoff areas from the same forest, and soil samples from an asymptomatic forest. The results show the reads distribution, the species abundance from each soil and the phylogenetic analyses. This study provides important insights into the *Phytophthora* species diversity in Mediterranean holm oak forests.

Introduction of *Phytophthora ramorum* in Fagaceae forests in Italy

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Since it became a quarantine pathogen for Europe, *Phytophthora ramorum* has been detected officially twice in Italian nursery stocks. Due to the low level of attention put in detection activities in the last years, presence of *P. ramorum* in Italian nurseries might be underestimated. Differently Italian *Fagaceae* forests have been largely inspected for *Phytophthora* presence especially in soil by means of classical baiting methods followed by morphological and molecular identification. Results of these activities never recorded the presence of *P. ramorum* in *Fagaceae* forests in Italy. Recent utilization of mass sequence techniques provided the possibility to analyse in one step, and high sensitivity, *Phytophthora* population in forest soils. Pyrosequencing analysis of chestnut soils has been carried out in two sites in Italy to evaluate the diversity of resident *Phytophthora* community. Sequence data have been analysed with dedicated database and resulted in a range of *Phytophthoras* including species known to be common in chestnut and beech forest soils in Italy. In addition some new species resulted to be present and represented by a discrete number of reads. Among these, *P. ramorum* was commonly detected in chestnut soils. To confirm the detection, DNA's utilised for pyrosequencing was amplified with species specific primers sets for *P. ramorum* and the amplicons obtained were sequenced. Sequences obtained matched with 100% identity with *P. ramorum* sequences on database. Cryptic presence of *P. ramorum* in forest soils would represent an important improvement of knowledge on epidemiology and invasion mechanisms of this species in Mediterranean climate.



SESSION 3

ECOLOGY, EPIDEMIOLOGY AND CLIMATE CHANGE



Ectomycorrhizae and *Phytophthora cinnamomi* relations within the rizosphere of *Quercus ilex*

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Oak decline is a major forest problem in Iberia and is principally associated with the loss of fine roots caused by *Phytophthora cinnamomi*. Ectomycorrhizae (ECM) are known to be beneficial for trees promoting plant growth and protecting roots from pathogen infections [1]. This study aimed to analyse the ECM community structure and its relation with the presence of *P. cinnamomi*. In 96 declining stands in Extremadura, Spain, three non-declining and three declining trees ($\leq 5\%$ and 21-40% of crown transparency, respectively) per stand were selected. For ECM and *Phytophthora* assessment monoliths of rhizosphere soil were collected from each tree. Each ECM tip was counted and categorized into morphotypes and the ECM abundance, species richness and diversity were estimated. Concerning physical and chemical soil factors, A soil horizon depth, soil bulk density, soil texture and pH, soil redox status and contents of ammonium (N-NH_4^+) and nitrate (N-NO_3) were measured. Preliminary results revealed a higher percentage of mycorrhizal root tips in non-declining trees than in declining trees ($62.3 \pm 17.8\%$ and $57.6 \pm 15.8\%$, respectively; $p < 0.05$). The declining status of trees had also an impact on species richness and Shannon diversity index, which were higher in non-declining trees ($p < 0.05$). The presence of *P. cinnamomi* was associated with an increase of fine root mortality ($p < 0.001$). Significant correlations were found between physical and chemical soil factors and ECM abundance. These results showed that crown transparency had a negative effect on ECM structure as demonstrated in other studies [2].

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Wildfire influences forest disease dynamics through selective host mortality and pathogen suppression: sudden oak death in Big Sur, CA

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Most disease ecology in forests has focused on shifts to community composition due to disease without consideration of the role of interacting disturbances. Sudden oak death (SOD), caused by *Phytophthora ramorum*, is associated with extensive tree mortality in coastal California forests. Wildfire is an important disturbance in these forests influencing community composition in the absence of SOD [1]. Fire may impact SOD directly through suppression of *P. ramorum* or indirectly through mortality of epidemiologically important hosts. Through surveys of burn severity, tree mortality, and regeneration following wildfires in SOD-impacted forests, we asked (i) how wildfire affected *P. ramorum* survival; and (ii) how forest recovery differs under the separate or joint influences of SOD and wildfire. Both disturbances cause selective mortality because the dominant tree species in these forests differ in their susceptibility to mortality from SOD and fire. In two habitat types, the dominant hosts for pathogen sporulation suffered greater fire-caused mortality than other species, which should lead to disease suppression in burned, infested areas relative to unburned, infested areas. We observed such suppression because only 20% of sampled, previously infested burned areas were found to contain *P. ramorum* immediately following the fire. In non-burned areas, forest composition has been shifting to



dominance by sporulating species that do not die from pathogen infection, leading to positive feedbacks on disease prevalence in these areas and continued mortality of canker hosts. The trajectory of post-disturbance recovery thus differs greatly among sites depending on the separate or joint influences of SOD and fire.

[1] Metz, M. R., Frangioso, K., Meentemeyer, R. K. and Rizzo, D. M. Interacting disturbances: wildfire severity affected by stage of forest disease invasion. *Ecological Applications*, **21** 313-320 (2011).

Susceptibility to *Phytophthora cinnamomi* of the main crops in dehesas and their influence in the epidemiology of the oak root disease

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Phytophthora cinnamomi, the main cause of *Quercus* root rot in south-western Spain, is an aggressive pathogen on *Lupinus luteus* (yellow lupin), causing root rot, wilting and death of this crop [1], but not on other crops (*Triticum aestivum*, *Avena sativa* or *Vicia sativa*) common in oak-rangeland ecosystems (*dehesas*) in the region. The pathogen was isolated from roots of wilted lupins in the field. Artificial inoculations on four cultivars of *L. luteus* reproduced the symptoms of the disease, both in pre- and post-emergence stages, recovering the pathogen from necrotic roots. Under controlled conditions and also in field samples, it was observed that *Lupinus luteus* increased the inoculum levels in the soil. For the rest of crops, by means of artificial inoculations with *P. cinnamomi*, positive isolations from infected roots of yellow lupin (symptomatic) and vetch (asymptomatic) were obtained, but never from wheat and oat (asymptomatic). Through *in vitro* infection experiments, it was demonstrated that yellow lupin highly stimulated the production of zoospores of *P. cinnamomi*. Vetch, wheat and oat did not stimulate zoospore production. In addition and opposite to lupin, vetch did not influence the viability of chlamydospores in the soil. We concluded that the culture of wheat, oat and even vetch in rangelands did not influence the epidemiology of *Quercus* root disease and they could be a good alternative to yellow lupin in rangeland ecosystems affected by root rot.

[1] Serrano, M. S., Fernández-Rebollo, P., De Vita, P., Carbonero, M. D. and Sánchez, M.E. *Lupinus luteus*, a new host of *Phytophthora cinnamomi* in Spanish oak-rangeland ecosystems. *European Journal of Plant Pathology* **128** 149-152 (2010).

Sudden oak death impacts to communities and ecosystems in California forests

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Recognition of *Phytophthora* importance has increased as more of these pathogens are introduced to naive host populations. We demonstrate epidemiological drivers of *Phytophthora* impacts on community composition, distribution of forest biomass, and ecosystem processes including litterfall, decomposition, and nitrogen cycling in California forests impacted by *Phytophthora ramorum* and the resulting disease sudden oak death. For *P. ramorum*, patterns of host mortality, pathogen spread, and accumulation of large woody debris are greatly determined by the prevalence of sporulation supporting species especially tanoak (*Notholithocarpus densiflorus*) which is rapidly killed following infection and California bay laurel (*Umbellularia californica*) which does not suffer deleterious impacts from infection. This leads to apparent competition (increased dominance of species not directly impacted by outbreak organisms) between the two species and positive feedbacks on pathogen populations in many *P. ramorum* impacted forests. *P. ramorum* caused mortality leads to modest changes in litterfall chemistry and soil N availability but the long-term consequences of species shifts are much larger and long-lasting



consequences to ecosystem processes. Control and eradication of *P. ramorum* is very difficult because of the pathogen's broad host range, survival in the environment, and prolific basal sprouting from disease-killed trees. The long-term consequence of this disease is removal of tanoak from the overstory in many forests, an impact similar to chestnut blight in the Northeastern USA. However, we found thresholds for pathogen persistence in epidemiological models suggesting a conservation strategy that combines identification of host resistance and management to reduce sporulation can retain biodiversity associated with tanoak.

Could climate warming be one of the causes of *Phytophthora alni* emergence in Europe?

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Emerging infectious diseases have become a major threat to the forest ecosystem conservation. Several factors may cause these emergences, among which evolution of silviculture practices, environmental changes, or introduction of alien pathogen by international trade. Those causes often can occur together and precisely assessing their importance is an important scientific question. In France, *Alnus glutinosa* is threatened by the development of the epidemic caused by *Phytophthora alni*. This pathogen is known to have been invasive in part of Europe and is the result of an interspecific hybridation event between *Phytophthora alni* subsp. *uniformis* and *Phytophthora alni* subsp. *multiformis*. The aim of this study was to assess whether the climate warming of the last decades might have participated determine in the disease emergence. For that, *P. alni* soil inoculum and incidence of the crown decline and of canker were monitored on 16 sites located along a altitudinal gradient in NE France used as an proxy for a temperature gradient. The results show that the disease incidence, i.e. the likelihood of new disease case in the sites, was positively correlated with the mean temperature of the winter. Evolution of past temperature in the last 40 years suggests that climate warming could by one of the cause explaining the emergence of *P. alni* alder decline.

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Influence of bird faeces in the behaviour of the root rot of *Quercus suber* caused by *Phytophthora cinnamomi* at Doñana Biological Reserve (SW Spain)

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Centennial cork oaks are considered keystone structures in the ecosystem of the stabilised sands of the Doñana Biological Reserve. These remnant big trees are currently threatened by nesting of colonial waterbirds, whose debris induced deep soil chemical changes. Since 2008 *P. cinnamomi* is also being isolated from roots and rhizosphere of declining trees [1]. *Phytophthora cinnamomi* has experienced a large spread in the Park over the last years, taking advantage of the extremely wet 2010 spring and winter. The objective of this work was to analyze the ability of the pathogen for oak root infection at various concentrations of natural and commercial (guano) bird dejections:

- a) *in vitro*, by testing the influence of three concentrations of bird faeces on clamydiospore viability, sporangial production and zoospore release, and
- b) *in planta*, adding bird faeces to infested soil at different concentrations and analyzing plant response



at the synergy between pathogen and dejections on infection of seedling roots.

The results obtained in the *in vitro* experiments showed that high concentrations of faeces inhibit crucial steps in the life cycle of the pathogen and consequently, could affect its infection ability. Results to be obtained in plant experiments will be show at the congress.

[1] De Vita, P., Serrano, M. S., Callier, P., Ramo, C., García L. V. and Sánchez, M. E. *Phytophthora* root disease: a new threat for cork oaks at Doñana National Park (South-Western Spain). *IOBC/WPRS Bulletin* **76** 93-96 (2012).

+ Poster 23

Spatial patterns of *Phytophthora cinnamomi* in declining Mediterranean forests: implications for tree species regeneration

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Soil-borne pathogens are a key component of the belowground community due to the significance of their ecological and socio-economic impacts. However, very little is known about the complexity of their distribution patterns in natural systems. Here we explored the patterns, causes and ecological consequences of spatial variability in the abundance of the soil-borne pathogen *Phytophthora cinnamomi* in Mediterranean forests, where this species represents a major driver of oak decline. We used spatially-explicit neighborhood models to predict *Phytophthora* abundance as a function of local abiotic conditions (soil texture) and the characteristics of the tree and shrub neighborhoods (species composition, size and health status). The implications of *Phytophthora* abundance for tree seedling performance were explored by conducting a sowing experiment in the same locations where pathogen abundance was quantified. *Phytophthora* abundance in the forest soil was not randomly distributed, but exhibited spatially predictable patterns influenced by both abiotic and particularly biotic factors (tree and shrub species). Soil texture seemed to affect *Phytophthora* abundance indirectly through its effects on soil water content, whereas woody species affected *Phytophthora* mostly directly by providing living host tissue with different susceptibility to pathogen attack. *Phytophthora* abundance reduced seedling emergence and survival, but not in all sites or tree species. Our findings suggest that heterogeneous spatial patterns of *Phytophthora* abundance at fine spatial scale can have relevant implications for the dynamics and restoration of declining Mediterranean forests.

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Roads and streams are not significant pathways for SOD spread in tanoak forests

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We used two approaches to study the importance of roads and streams as pathways of spread of sudden oak death in Oregon. A GIS-based spatial analysis of infected sites relative to the road and stream networks was compared to analysis based on a set of random points. Second, ground surveys of roads and streamside vegetation in the infested area mapped the association between *P. ramorum* in streams or on road surfaces and infection of adjacent vegetation.

The spatial analysis showed no association between roads and SOD sites. The median distance from *P.*



ramorum infections to roads was not significantly different from the median distance expected under randomness. In road sampling, *P. ramorum* was not recovered from roads or susceptible plants growing within splash distance from roads except when they were growing immediately beneath an infected over story tree. On the other hand, there was a significant association between *P. ramorum* in tanoak and proximity to streams. Surveys of streamside vegetation, however, produced no evidence of infection originating from stream-borne inoculum.

Results indicate that roads are not important dispersal pathways for *P. ramorum* in Oregon. This is probably testament to the effectiveness of the sanitation protocols incorporated in the SOD eradication program, as well as evidence that the harsh road environment does not favor *P. ramorum* survival. The positive correlation between SOD and streams apparently results from channeled air movement, carrying aerially dispersed sporangia up and down stream valleys, not from *P. ramorum* transported in stream water.

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Nature of *Phytophthora* inoculum in flowing surface waters

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Phytophthora species are regularly recovered from surface waters, often in the absence of apparent sources of inoculum [1, 2]. The persistence of such plant pathogens in water bodies has significant implications for their spread and management in agricultural as well as more natural contexts. To determine if *Phytophthora* spp. can complete their life cycle in aquatic environments, we have undertaken experiments focused on *P. ramorum* and *P. gonapodyides* in California coastal streams. Exposing fresh rhododendron leaves along with those killed by drying or freezing to inoculum in naturally infested streams and in controlled environment experiments indicated that *P. ramorum* has a limited ability to colonize degraded leaf litter in aquatic environments. In contrast, *P. gonapodyides* more readily colonized dead leaves. The potential of aquatic and riparian plants as sources of *Phytophthora* inoculum in aquatic environments will be addressed by surveys for cryptic infections. Colonies of *P. ramorum* and *P. gonapodyides*-like spp. were recovered from glass slides exposed in streams, indicating that propagules passively adhere to substrates in streams. Colonies were also recovered from leaves and glass slides suspended in tubes in stream flow, indicating that propagules adhere without being trapped between surfaces. To determine the presence of sporangia, zoospores and cysts in flowing stream water, selective isolation of propagule types will be based on differences in size and lack of a cell wall in zoospores.

[1] Hong, C. X. and Moorman, G. W. Plant pathogens in irrigation water: Challenges and opportunities. *Crit. Rev. Plant Sci.* **24** 89-208 (2005).

[2] Reeser, P. W., Sutton, W., Hansen, E. M., Remigi, P. and Adams, G. C. *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia* **10** 22-35 (2011).



Seasonal variation of inoculum density and species composition of soilborne Phytophthoras in an infected black walnut stand in Hungary

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Previous studies showed the presence of *Phytophthora plurivora* and *Phytophthora cactorum* in the soil of declining black walnut stands in Hungary, and the pathogenicity of these species was proved by inoculation of seedlings [1].

The health condition of a 73 years black walnut stand was examined in June and September 2011 in West-Hungary.. The stand is situated on a drained floodplain. The trees showed declining symptoms: sparse crown, drying branches, small leaves with yellowish discolouration. Twenty trees were selected for monitoring survey. The health condition of the trees was evaluated on a 4-pointed scale. Soil samples were collected from the rhizosphere of each of examined trees for isolation of Phytophthoras. *Phytophthora* species were isolated on selective agar media, using the leaf baiting method (*Rhododendron* and *Prunus laurocerasus* leaves as baits). The spots on the baits were counted to estimate the inoculum density in the soil sample. The isolates were identified by morphological and molecular methods. The morphological features of the isolates were examined on cultures grown on carrot agar at 20 °C. The molecular identification was performed by sequencing the ITS 1 and ITS 2 region of the rDNA of selected isolates.

The healthy state of 30% of the investigated trees got worse during the summer. However, the inoculum density and the isolation success were lower in September in almost every soil samples. There were changes also in the species composition: In June 59.26% of the isolates were *Phytophthora cactorum* and 18.52% *Phytophthora plurivora*, however, 100% of the isolates were *Phytophthora plurivora* in the collection of September.

[1] Szabó, I. and Lakatos, F. *Phytophthora* species isolated from declining forests in Hungary. *Növényvédelem*, **44** 607-613 (2008).

Epidemiology of *Phytophthora ramorum* and *Phytophthora kernoviae* on *Vaccinium* in the natural environment in the UK

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Phytophthora kernoviae was first detected causing stem blackening and leaf necrosis on *Vaccinium myrtillus* in late 2007 in woodland in the South West of England. Further UK wide surveys have since detected the pathogen in 12 additional locations, mainly in the South West of England. *Phytophthora ramorum* was confirmed infecting *vaccinium* in late 2008, causing similar symptoms to those seen by *P. kernoviae*, and to date has been recorded at 10 sites, predominantly in the West Midlands and Wales. Differences can be seen in the habitats favoured by the two pathogens with *P. kernoviae* mainly infecting *vaccinium* in heathland, whilst *P. ramorum* infections mainly occur in *vaccinium* growing in a woodland environment. Although the total number of sites infected with either pathogen is small, the number of confirmed new sites has been increasing year on year.

Laboratory experiments have been carried out in order to determine the relative susceptibility of *V. myrtillus* to varying spore concentrations of both *P. kernoviae* and *P. ramorum* and how this compares



to other host species including rhododendron and viburnum. In order to establish if there are periods of high host susceptibility bait plants were positioned in *P. kernoviae* diseased areas in both heathland and woodland along with a datalogger recording temperature and humidity. Spore washes were also carried out to determine timing of sporulation. Monitoring over 2 years has shown *vaccinium* is susceptible all year with peaks in infection and sporulation coinciding with periods of high humidity/rainfall.



SESSION 4

RESISTANCE, PATHOGENESIS, ECOPHYSIOLOGY



Blocking of α -plurivorin compromises *Phytophthora plurivora* pathogenicity towards *Fagus sylvatica* seedlings

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To manipulate host metabolism during infection, *Phytophthora* species secrete many effectors, including high amounts of elicitors, a small protein family first described to elicit defence responses in tobacco plants. Elicitors trigger a variety of defence responses, including programmed cell death (PCD) in several plants, sharing many features of pathogen-associated molecular patterns (PAMPs). However, the precise role of elicitors as a virulence factor has yet to be clarified.

Here, we show that α -plurivorin, an elicitor secreted from *Phytophthora plurivora*, is essential for virulence and correlated with pathogen penetration in the host root tissues and defence suppression in beeches.

The blocking of α -plurivorin by incubation with a specific antibody during infection drastically impaired its internalization in host tissues and *P. plurivora* penetration, disabling the pathogen's disease promotion in beech seedlings. Furthermore, the lack of α -plurivorin inside the host tissues led to an up-regulation of defence-related genes, suggesting that α -plurivorin acts as a defence suppressor during infection. All of the infected plants treated with the anti- α -plurivorin antibody survived whereas most of the other infected plants died by the end of the experiment. Remarkably, given the potential of hundreds of effector genes in the *P. plurivora* genome, inhibition of α -plurivorin compromises *P. plurivora* pathogenicity, suggesting that α -plurivorin is essential for virulence.

Because elicitors are ubiquitously secreted by *Phytophthora* species, it is very likely that these molecules can also act as virulence factors in other *Phytophthora*-susceptible plant interactions. Therefore, the selective blocking of elicitors function might be a specific target for protecting plants against *Phytophthora*.

Identification of *Phytophthora cinnamomi* gene transcripts in infected cork oak roots

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Phytophthora cinnamomi is associated with the severe decline that is threatening typical agroforestry ecosystems with an overstorey of cork (*Quercus suber*) and holm (*Q. ilex*) oaks, resulting in significant economic and ecologic losses. The transcriptome analysis of *Q. suber* directed to genes related to biotic stress caused by *P. cinnamomi* infection is presently in progress in our laboratories as part of the Cork Oak ESTs Consortium programme (<http://coec.fc.ul.pt/>).

Cork oak roots were immersed in a *P. cinnamomi* zoospore suspension and incubated for 8, 20, and 36 h. RNA was extracted and pooled and cDNA was synthesised. cDNA was fragmented, the sequencing adaptors ligated and pyrosequenced using 454 GS FLX Titanium (Roche-454 Life Sciences) technology. Reliable reads were assembled and the resulting fasta files were run in the Blast2GO application



(<http://www.blast2go.org>). Blast2GO is an all in one tool for Functional Annotation (FA) of sequences and the analysis of annotation data. The FA was run in 3 steps: BLAST to find homologous sequences [queries against the NCBI Databases using BLASTx], MAPPING to retrieve Gene Ontology terms and ANNOTATION to select reliable functions. Different annotation databases were used: GO, Enzyme Codes, InterPro and KEGG.

More than 580 *Phytophthora* contigs were identified and their Functional categories will be presented. Transcripts putatively involved in pathogenicity will be disclosed. The project sponsored by the DOE Joint Genome Institute for the sequencing of the whole genome of *P. cinnamomi* was completed in March 2012. Upon its public release, it will be possible to access information on the complete sequences of the *P. cinnamomi* genes expressed following infection of cork oak roots.

Transcriptome analysis of *Quercus suber* roots in response to *Phytophthora cinnamomi*

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The oomycete *Phytophthora cinnamomi* is widely distributed in Iberian soils and causes root rot on *Q. suber* and *Q. rotundifolia*. The EST analysis of *Q. suber* directed to genes responsive to *P. cinnamomi* infection is presently in progress in our laboratories as part of the Cork Oak ESTs Consortium programme (<http://coec.fc.ul.pt/>).

Cork oak roots were immersed in a *P. cinnamomi* zoospore suspension and incubated for 8, 20, and 36 h. Non treated roots were used as controls. RNA was extracted from healthy (RC) and infected (RIZ) roots and cDNA was pyrosequenced using 454 GS FLX Titanium (Roche-454 Life Sciences) technology. Adapter and polyA clipped reads were submitted to *de novo* assembly.

Only contig sequences where at least 10 reads were clustered together were considered for quantification purposes. Expression values of contigs in the RIZ and RC samples were obtained in RPKM (Reads Per Kb exon (contig) per Million mapped reads) and were directly comparable to each other. A ten-fold difference in the RPKM expression values was used as a clear cutoff for differential expression. Contigs showing over/under expression or presence/absence were selected for further analysis with the Blast2GO application (<http://www.blast2go.org>).

195 contigs were found to be over-expressed in RIZ and 85 under-expressed; 1771 were only present in RIZ and 1606 were only present in RC.

Blast2GO allowed to assign ontology classes to genes over expressed or only present in infected roots. In the biological process category, genes function categories associated with biosynthetic, catabolic, primary and secondary metabolic processes, response to stress and to biotic stimulus are represented in the transcriptome. In the molecular function category the most highly represented category includes genes involved in kinase and transferase activities and in the cellular component category, genes function categories associated with cell wall, cytoplasm and plasma membrane are the most represented.

Overall, we conclude that *Q. suber* respond to *P. cinnamomi* infection by activating resistance responses.



Quantitative trait loci for resistance to *Phytophthora cinnamomi* in two host species

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Castanea sativa and *Quercus robur* are two ecological and economical important European species. Both are host for *Phytophthora cinnamomi*, the causal agent of oak or chestnut ink disease. Sources of resistance to ink disease were identified in *C. mollissima* and *C. crenata* and susceptibility to *P. cinnamomi* is varying in *C. sativa* and *Q. robur* [1]. However, very little is known about the genetic and the evolution of this adaptive trait in Fagaceae species.

Knowing the macrosyteny and macrocolinearity occurring between the two genera [2] our objective was to compare the genetic architecture of resistance to *P. cinnamomi* trait in oak and chestnut. We will then test the hypothesis that the synteny can allow us to use the *Quercus* candidate genes involved in resistance to pathogens as putative candidate genes in *Castanea*.

Components of genetic resistance to *P. cinnamomi* were investigated in a full-sib family of *Q. robur*. For chestnut, two progenies from two controlled crosses (*C. sativa* x *C. crenata* and *C. sativa* x *C. mollissima*) were used. The three progenies were vegetatively propagated by cuttings. Resistance to ink disease was estimated in glasshouse by inoculating *P. cinnamomi* on the cuttings stems and by measuring the length of the induced lesion. The experiments were repeated two successive years.

Nine quantitative trait loci (QTL) involved in *P. cinnamomi* resistance mechanisms were located on eight linkage groups of the oak parental genetic maps, explaining 4 to 9 % of the phenotypic variation. For chestnut, QTL are under investigation (ongoing project).

[1] Robin, C., Morel, O., Vettrano, A.M., Perlerou, C., Diamandis, S. and Vannini, A. Genetic variation in susceptibility to *Phytophthora cambivora* in European chestnut (*Castanea sativa*). *For. Ecol.Man.* **226**199-207 (2006).

[2] Barreneche, T., Casasoli, M., Russell, K., Akkak, A., Meddour, H., Plomion, C., Villani, F. and Kremer, A. Comparative mapping between *Quercus* and *Castanea* using simple-sequence repeats (SSRs). *Theor. Appl. Genet.* **108** 558–566 (2004).

Interactions between *Phytophthora cinnamomi* and *Phlomis purpurea*, a plant resistant to the pathogen

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Phlomis purpurea is spontaneous in *Quercus suber* and *Q. ilex* forest habitats in southern Portugal growing in areas affected by the decline disease. As previously described, *in vitro* inhibition of the oomycete life cycle structures by *P. purpurea* crude root extracts (PRE) at 10 mg ml⁻¹, ranged from 85% to 100% [1]. Recently, we have shown *in planta* that, at the same concentration, PRE significantly



inhibited the infection of *Q. suber* roots by *P. cinnamomi*. Moreover, PRE appear to elicit a defence response: radicles of two-week-old *Q. suber* exposed to PRE at 10 mgml⁻¹, 24 h prior to zoospore challenge were significantly protected from infection.

Cyto-histological evaluations are being made to elucidate:

1. How *P. purpurea* manages to avoid infection by *P. cinnamomi* zoospores;
2. How PRE inhibits the infection of *Q. suber* by zoospores;
3. Whether PRE are elicitors.

Control and inoculated roots at 0, 6, 24, 48 and 72 hours post inoculation (hpi) for *P. purpurea* and at 0 and 48 hpi for *Quercus suber* are being analysed, by light microscopy. Preliminary results showed the pathogen does not penetrate the plant rhizodermis, suggesting a type I resistance (the first time, to our knowledge it is observed in respect to *P. cinnamomi*). These features make this plant interesting to study its interactions with the oomycete and to explore it in a biocontrol perspective. A fraction isolated from PRE, showed a 100% *P. cinnamomi* inhibition at 0.5 mg ml⁻¹. Its major compound, m/z 473, was separated from contaminants. Its structure determined by MS and NMR will be presented.

[1] Neves, D. (2007). In vitro activity of plant crude extracts and in situ protective effect of *Phlomis purpurea* against *Phytophthora cinnamomi*. MSc dissertation. Cranfield University.

How do *Phytophthora* spp. harm woody plants?

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One of the objectives of working group 2 “Host-pathogen- interactions” within the FPC Cost action FP0801 (“Established and Emerging *Phytophthora*: Increasing Threats to Woodland and Forest Ecosystems in Europe”) was to analyze susceptible and resistant *Phytophthora* host interactions and shed light on the question “how do *Phytophthora* spp. may harm woody plants”. Our evaluation comprised thirteen worldwide distributed species of the genus *Phytophthora* with the potential to invade at least nineteen different woody plant species.

Different conceptual models describing the primary infection of roots, trunks or of leaves were developed, based on extensive literature review and group discussions. We aimed to figure out which plant organs are infected first and in which tissues the mycelium grows during the early and late infection stages.

The significance of host-root exudates and their specific components to attract *Phytophthora* zoospores is of great interest. In order to understand local and systemic responses, different physiological and biochemical reactions of host plants triggered by pathogen attack were evaluated.



Molecular studies completed the synopsis, focusing especially on virulence and avirulence genes of the pathogens as well as on the components of *Phytophthora* secretomes such as PAMPS and effector molecules. In this context the significance of *Phytophthora* elicitors to establish a susceptible host-pathogen interaction or to act as elicitor is discussed.

Multitrophic interactions between *Quercus robur*, *Phytophthora quercina* and *Piloderma croceum* (on a joint experimental platform)

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Within the joint research project “TrophinOak”, we analyze multitrophic interactions of *Quercus robur* micro-cuttings DF159 with respect of the rhythmic growth of oak. Our research team focuses on interactions with the root pathogen *Phytophthora quercina*. In addition, we compare the effects of the ectomycorrhizal (EM) fungus *Piloderma croceum* F 1598, as an additional interacting partner.

Earlier conceptual models described the effects of alternating root and shoot-flushes of oaks on the susceptibility against *P. quercina* [1]. It was postulated, that ongoing shifts of resource availability towards flushing shoots should render the non-flushing roots less capable of repair and defense and therefore more susceptible against *P. quercina*.

Research on *P. croceum* revealed a protective effect of EM against root pathogens, possibly due to chemical and/or mechanical shielding. Further studies demonstrated the prevalent role of alternating root/shoot-growth of oaks for this EM formation [2].

The oaks were cultured in axenic soil-systems either single- or co-inoculated with interaction partners. ¹⁵N-/¹³C stable-isotope labeling was applied and the oaks, expressing root- or shoot-flush, were harvested. Subsequently, we quantified *Phytophthora*-infection, analyzed resource allocation by isotopic-tracing as well as by analysis of soluble sugar and starch, and measured gene-expression patterns in oaks using Illumina high throughput sequencing.

Our data revealed significant differences between treatments and growth-stages concerning resource-allocation and susceptibility against *P. quercina*. Although investigations are still ongoing, the above hypothesis that higher C availability in roots reduces susceptibility to *P. quercina* has to be rejected for our system.

[1] Jönsson, U. A conceptual model for the development of *Phytophthora* disease in *Quercus robur*. *New Phytol.*, **171** 55–68 (2006).

[2] Branzanti, M.B., Rocca, E. and Pisi, A. Effect of ectomycorrhizal fungi on chestnut ink disease. *Mycorrhiza* **9** 103–109 (1999).

Drought, fire and flood: approaches to the renewed challenge of *Phytophthora cinnamomi* in south-eastern Australia

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For the years 2000–2010 south-eastern Australia experienced the driest climatic period on record. In 2006 devastating bushfires raged across the state of Victoria and again in 2009 resulting in Australia's greatest loss of life from bushfire. Since late 2010 there have been episodic and catastrophic floods. *Phytophthora cinnamomi* and the disease that it causes in native vegetation have not been abated by these increasingly harsh environmental conditions. We now have evidence of renewed, widespread and



severe impacts of the pathogen on natural systems. Many of the areas in Victoria for which *P. cinnamomi* has been recorded [1] have been affected by drought and fire and recent high rainfall events have stimulated large disease outbreaks in many national parks and reserves. In collaboration with government agencies we are undertaking an extensive surveillance and monitoring program in key biodiverse regions in the state with the aim of prioritising management. Work is continuing at the molecular level to unravel the basis of resistance [2] in a range of host species, for example, we have good evidence for the involvement of specific resistance-related signalling pathways in roots of the model, *Zea mays*. This finding may give us leads into ways in which we can modify or engineer resistance to *P. cinnamomi* in susceptible plants. We have also identified a unique signalling phospholipase from *Phytophthora* that may explain the lack of phospholipase C in the genomes of the three sequenced species. We are currently investigating this protein as a possible target for antibiotics.

[1] Cahill, D. M., Rookes, J. E., Wilson, B. A., Gibson, L. and McDougall, K. L. *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. *Aust. J. Bot.* **56** 279-310 (2008).

[2] Allardyce, J. A., Rookes, J. E. and Cahill, D. M. Defining plant resistance to *Phytophthora cinnamomi*: A standardised approach to assessment. *Journal of Phytopathology*, Doi:10.1111/j.1439-0434.2012.01895.x (2012).



SESSION 5

SURVEYS, DIVERSITY, INVASION AND SPREAD (cont.) / CLASSICAL TAXONOMY AND CLASSICAL MOLECULAR DIAGNOSTICS



6th IUFRO Working Party 7.02.09
“*Phytophthora* in Forests and Natural Ecosystems”
9th-14th September 2012
Córdoba-Spain

***Phytophthora* species associated with *Alnus rubra* in western Oregon riparian ecosystems**

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A survey of alder health was conducted from 2010-2012 in western Oregon USA with particular attention to *Phytophthora* species associated with decline. Water, bark, soil, and root samples were collected systematically. Water samples were filtered, soil samples were baited, and root samples were washed and surface sterilized. All samples were plated on *Phytophthora* selective media. Specifically, necrotic margins, cankered tissue, and water soaked regions of above ground bark and below ground roots were plated. To date, only *Phytophthora siskiyouensis* has been recovered from above ground alder bark. *Phytophthora alni uniformis*, *Phytophthora siskiyouensis*, and other *Phytophthora* species from ITS clades 2, 6 and 7 were found in symptomatic root tissue. More *Phytophthora* species have been recovered in water samples compared to *Phytophthora* species recovered from alder roots. Preliminary pathogenicity testing suggests both *P. alni uniformis* and *P. siskiyouensis* are able to cause small cankers. More extensive pathogenicity tests are currently underway. Observation and culture evidence suggest *Phytophthora* species are correlated with decline observed in patches along waterways. There is no evidence *Phytophthora* species found are invasive, but infected trees may provide better habitat for insects and secondary pathogens.

Poster 51

***Phytophthora ramorum* and *P. lateralis* in Northern Ireland**

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Phytophthora ramorum was first detected in Northern Ireland on plants in trade in 2004. The first outbreak on plants in the wild was in 2006 on rhododendron in private gardens and unmanaged woodlands on private estates. The first outbreak of *P. ramorum* on larch was in 2010. A strategy of eradication and containment has resulted in the felling of 300+ ha of larch. Aerial surveys of N. Ireland have given no evidence that the pathogen has spread to the west. Significant improvements have been made on both the extraction of *P. ramorum* DNA from wood and its amplification which has very significantly improved detection sensitivity. Isolates of *P. ramorum* obtained from rhododendron, larch, oak and *Vaccinium* have been shown to belong to a new lineage of *P. ramorum* [1]. The epidemiological significance of this new lineage is currently being investigated.

In August 2011 *P. lateralis* was first diagnosed on specimen arboretum trees of Lawson cypress (*Chamaecyparis lawsoniana*) growing in a large public forest park in the southern part of Northern Ireland. It was subsequently found in another public park in the north as well as in two large Lawson cypress forest plantations. A number of individual trees or groups of trees, mainly in private gardens have been found to be infected. Studies are currently being undertaken to determine the factors influencing the survival and spread of the pathogen which will in turn inform the most appropriate disease control and biosecurity measures to be taken.

[1] Poucke, K., Franceschini, S., Webber, J. F., Vercauteren, A., Turner, J., McCracken A. R., Heungens, K. and Brasier C. M. Discovery of a fourth evolutionary lineage of *Phytophthora ramorum*: EU2. (In preparation) (2012).



***Phytophthora bilorbang* prov. nom., a new species associated with declining *Rubus anglocandicans* (blackberry) in Western Australia**

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Rubus anglocandicans is the most widespread and invasive species in the *Rubus fruticosus* aggregate (European blackberry) found in Australia [1]. Blackberry has been targeted by biological control since the 1980s and most of this effort has focused on introducing exotic strains of the host-specific leaf rust, *Phragmidium violaceum*. During surveys established to assess the releases of the rust fungus in 2005, dead and diseased blackberry plants were found at two locations along the Warren and Donnelly Rivers in the Manjimup region of Western Australia (P. Yeoh and L. Fontanini personal communication). The disease symptoms could not be attributed to the rust fungus and the phenomenon has been referred to as 'blackberry decline'. The disease appears to be due to root pathogen(s) and during initial sampling several *Phytophthora* species were isolated. In order to investigate the cause(s) of disease and the potential role of *Phytophthora* species in the decline, field surveys were carried out over 2010 and 2011 in the decline and non-decline sites along the Warren and Donnelly Rivers. During these surveys, *P. taxon oaksoil* was recovered from decline sites. Several isolates of this taxon have been isolated from Europe [2], and given a provisional name (oaksoil) until formal description. This taxon is described here as *Phytophthora bilorbang* prov. nom.; a new taxon within the ITS Clade 6, sub-clade II of *Phytophthora*. This is the first report of this new *Phytophthora* species in association with declining *R. anglocandicans*.

[1] Evans, K. J. and Weber, H. E. *Rubus anglocandicans* (Rosaceae) is the most widespread taxon of European blackberry in Australia. *Aust. Syst. Bot.*, **16** 527–537 (2003).

[2] Brasier, C. M., Cooke, D. E. L., Duncan, J. M. and Hansen, E. M. Multiple phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides*–*P. megasperma* ITS Clade 6, which tend to be high- temperature tolerant and either inbreeding or sterile. *Mycological Research*, **107** 277–290 (2003).

Spatial patterns of holm and cork oak decline in Extremadura, Spain

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Nowadays Phytophthora epidemic, which causes oak decline, represents one of the major threats for Iberian forest putting at risk its environmental, economical and cultural wealth. In order to develop rational strategies to prevent and control this forest disease, knowledge of coarse-scale disease dynamics in space, time and severity dimensions is needed. The aims of this study are 1) to know where and how much oak decline disease there is in Extremadura, 2) to establish a current baseline to measure control effort efficiency and 3) to identify risk factors. A 10% of regional area was sampled randomly picking quadrangles of aerial infrared ortho-photography. IR digital imagery was interpreted by trained operators to find out and delineate oak decline symptomatic foci. Preliminary results point out that more than 1% of holm and cork oak forests of Extremadura exhibit symptoms of decline. These foci were completely spread over regional oak forest area with a geographic pattern that matches with the isolation record of *Phytophthora cinnamomi*. The number of disease plots increased from 470 reported circa 2000 [1] to more than 4000 symptomatic points estimated in this work, a ten fold increase in a decade. Secondary infections originated recently could be explaining the observed pattern consisting on clusters of small size foci. Finally, disease foci were found more frequently in areas with signs of



livestock heavy trampling or in zones near water.

[1] Del Pozo, J.L. Prospección de la seca en Extremadura. Análisis de los resultados. In: Gestión Ambiental y Económica del Ecosistema Dehesa en la Península Ibérica (M. Espejo, M. Martín, C. Matos, S.J. Mesías, eds.), Junta de Extremadura, Mérida. pp. 131-143 (2006).

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***Phytophthora* species in Serbia**

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Since there is a great economic and ecological threat posed by *Phytophthora* species, a study has been performed during the years 2009-2012, aiming at determining the presence and diversity of *Phytophthora* species in both natural ecosystems and amenity trees in Serbia.

Sampled trees showed symptoms typical of *Phytophthora* infections, such as presence of collar rots or stem cankers with dark exudates, chlorosis and wilting of leaves, increased crown transparency, dieback, dying of shoots, branches or parts of the crown, root lesions, and decay and loss of fine roots. Sampling and isolation methods were according to Jung (2009) [1] and Jung et al. (1996) [2]. Tissue samples were taken from necrotic parts and plated directly onto selective agar medium (V8A-PARPNH). Soil containing fine roots was sampled in the form of soil monoliths, measuring ~ 25x25x25 cm, and isolation tests were performed using oak, beech and cherry laurel leaves as baits. Both symptomatic and healthy trees were sampled. In total 167 samples were taken from 26 different host species including *Quercus robur*, *Q. petraea*, *Q. cerris*, *Fagus sylvatica*, *Fraxinus angustifolia*, *Acer pseudoplatanus*, *A. platanoides*, *A. heldreichii*, *Populus* spp., *Juglans regia* and *Betula pendula*, in both forest ecosystems and amenity tree stands. *Phytophthora* species were isolated from about 68% of samples. Also, many isolates of *Pythium* spp. were obtained.

After a detailed morphological and molecular identification of all isolates, nine different *Phytophthora* species have been confirmed, i.e. *P. europaea*, *P. cambivora*, *P. citricola*, *P. cactorum*, *P. plurivora*, *P. polonica*, *P. quercina*, *P. taxon 'Pg chlamydo'*, and *P. lacustris* (previously known as *P. taxon 'Salixsoil'*), and some of them were recorded for the first time in different ecosystems in Serbia.

[1] Jung, T., Beech decline in Central Europe driven by the interactions between *Phytophthora* infections and climatic extremes. *Forest Pathology* **38** 73-94 (2009).

[2] Jung, T., Blaschke, H., Neumann, P. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology* **26** 253-272 (1996).

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A species concept for *Phytophthora* taxon Agathis (PTA) — causal agent of root and collar rot of *Agathis australis* in New Zealand¹

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Kauri Dieback has been identified as an increasing problem affecting kauri (*Agathis australis*) across the Auckland and Northland regions. *Phytophthora* taxon Agathis (PTA) has been identified as a causal agent of a root and collar rot of kauri [1]. 'PTA' shares a place in *Phytophthora* ITS Clade 5 [2] with *P. heveae* and *P. katsurae*. PTA was originally misidentified as the morphologically similar *P. heveae*. It has been established that PTA has a different oogonial morphology to both *P. heveae* and *P. katsurae*. The sequencing of eight loci from both the nuclear and mitochondrial genomes has been used to resolve the species boundaries within Clade 5. Bayesian inference phylogenies reveal PTA is a discrete taxonomic entity, separate from either *P. katsurae* or *P. heveae*. Further, because of its unique colony morphology, oogonial characters, persistent sporangia and pathogenicity to *Agathis australis* we recognise PTA indet. as a new species in ITS Clade 5 of the genus *Phytophthora*.

[1] Beever, R.E., Waipara, N.W., Ramsfield, T.D., Dick, M.A. and Horner, I.J. Kauri (*Agathis australis*) under threat from *Phytophthora*? Proceedings 4th IUFRO Working Party 7-02-09 "Phytophthora in Forests and Natural Ecosystems", 26-31st August 2007, Monterey, California, USA (2008).

[2] Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. and Brasier, C.M. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Gen.Biol.*, **30** 17-32 (2000).

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Phytophthora ITS Clade 3 expands to include a sixth new species, *P. taxon pluvialis*

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Phytophthora ilicis was described in 1957 as a pathogen of holly (*Ilex aquifolium*). In 2002 it was one of two founding members of ITS Clade 3. In 2002–2003 three new species were added. We propose to add a sixth species to be named *P. pluvialis*. While exhibiting unique features, *P. taxon pluvialis* shares some morphological features with other members of Clade 3, excepting *P. quercina*. It has ovoid, partially caducous, semi-papillate sporangia with variable length pedicels borne on unbranched or simple sympodial sporangiophores. Antheridia are predominately amphigynous. Oogonia measure around 32 µm diameter. All isolates studied have identical nuclear ITS sequence, with at least seven different 'genotypes' recognized by mitochondrial *cox* spacer sequences. Compared with other species in Clade 3, DNA sequences form a unique phylogenetic taxon. *P. taxon pluvialis* has been recovered mostly from streams, soil and canopy drip in the mixed tanoak (*Notholithocarpus densiflorus*)-Douglas fir (*Pseudotsuga menziesii*) forest in Curry County, Oregon, USA. It has been found only rarely associated with twig and stem cankers on tanoak. Like *P. pseudosyringae* and *P. psychrophila*, *P. taxon pluvialis* is



not strongly host-associated, and may be endemic. This contrasts with *P. ilicis*, which has been found only causing disease on holly, and with *P. nemorosa*, which is strongly associated with disease on tanoak and California bay (*Umbellularia californica*).

In memory of Dr Ross E. Beever, 1946–2010.

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***Phytophthora acerina* sp. nov., a new species from the *P. citricola* complex causing aerial cankers on *Acer pseudoplatanus* in Italy**

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A new homothallic *Phytophthora* species with paragynous antheridia, semipapillate, persistent and highly variable sporangia, and optimum and maximum temperatures of 25 and 32 °C, respectively, was consistently isolated from aerial bleeding cankers and rhizosphere soil of declining *Acer pseudoplatanus* trees in planted forests in northern Italy. In underbark inoculation tests all isolates were highly aggressive to *A. pseudoplatanus* and *Fagus sylvatica* indicating that this pathogen might pose a serious threat to maple and beech forests in Europe. All isolates share identical ITS and *cox1* sequences and represent a distinct subclade of the *P. citricola* complex. Interestingly, all isolates showed a high abortion rate of the oospores distinguishing this taxon from all other known species and taxa of the *P. citricola* complex. Most likely, this species evolved under conditions that did not require oospores as long-term resting structures so that selection did not weed out deleterious mutations in the breeding system. A gradual loss of fertility in favour of a continuous asexual reproduction by zoospores is well-known from *Phytophthora* species in wet or aquatic habitats. Due to its unique combination of morphological, physiological and molecular characters this new taxon is currently being described as a new species, *P. acerina* sp. Nov.



SESSION 6
RESISTANCE, PATHOGENESIS, ECOPHYSIOLOGY (CONT.)



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A new method to quantify zoospore chemo-attraction

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Phytophthora species release zoospores which are, in case of soil born species, attracted by root exudates, before they encyst and infect root tissue. To test the activity of zoospores to several root exudates and to quantify zoospore attraction we developed a simple, cheap and easy-to-built new device. It consists of three cylindrical plastic containers A, B and C (length 11.5 cm, diameter 3 cm), that are connected to each other at the bottom with two tubes (length 1cm, diameter 0.5 cm) holding a dialysis membrane in the middle position. Reservoir A can be filled with the control (water, buffer) and C with the test solution (e.g. root exudate). Flask B in the middle holds the zoospore suspension. The zoospores swim towards the attracting solution and are finally trapped at the dialysis membrane where they encyst. The activity of different zoospore attractants can be compared and quantified by counting the zoospores cists on the membrane under the microscope or by quantitative real-time PCR in combination with *Phytophthora* primers. Using this zoospore trap, we tested the attraction of zoospores of *P. plurivora* and *P. nicotianae* towards root exudates of their hosts. qRT-PCR data showed that *P. plurivora* zoospores were attracted 20.000 times more to root exudates of *F. sylvatica* as compared to distilled water. Citrus Sunki (susceptible) root exudates attracted 6 times more zoospores of *P. nicotianae* than did Swingle (resistant).

This new trap can easily be built with more than two containers connected to the zoospores reservoir (B), so that it is possible to compare differential attraction of several root exudates as well as of single compounds regarding their activity on zoospore attraction. This would contribute with important information about the early interactions in the rhizosphere.

Fluorescent *in situ* hybridization (FISH) assay as a tool to microscopically view *Phytophthora cinnamomi* growth within plant tissues

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The microscopic examination of naturally infected plant material for the presence of *Phytophthora cinnamomi* can be problematic as structures such as hyphae, hyphal swellings, chlamydospores, and oospores are often indistinguishable from those of other oomycetes or fungi. Frequently, it would be useful to be able to clearly differentiate *P. cinnamomi* from other micro-organisms, especially when trying to determine how the pathogen is surviving in plant material particularly in harsh environments. Consequently, the lack of stains that can clearly and definitively localise hyphae and reproductive structures of *P. cinnamomi* within plant material is a limitation in increasing our understanding of the biology of the pathogen in susceptible and tolerant plant species in different ecosystems. This study demonstrates that a *P. cinnamomi* specific, fluorescently labelled DNA probe can be used to specifically detect and visualise *P. cinnamomi* in plant material using fluorescent *in situ* hybridization (FISH) without damage to plant or pathogen cell integrity. The method will allow us to



more accurately study plant-*P. cinnamomi* interactions in plants, and to be particularly useful in naturally infected material.

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Histological changes of *Quercus ilex* seedlings infected by *Phytophthora cinnamomi*

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Since the early 1980s, severe decline and occasional sudden death of extensive areas of holm oak (*Quercus ilex*) and cork oak (*Q. suber*) woodlands in the southwest of the Iberian Peninsula have been associated to the damage caused by *Phytophthora cinnamomi*, an alien and invasive pathogen infecting roots of many woody species. The objective of this work was to perform histological studies of holm oak infected by *P. cinnamomi*. To achieve this, *Q. ilex* subsp. *ballota* acorns were collected in Extremadura (Spain) and germinated in sterile vermiculite. Five-week-old seedlings were inoculated by immersing them in flasks with *P. cinnamomi* colonised agar and soil extract solution. Root sections were obtained every 12 hours, during 7 days. Root tissue was fixed with FAA and Karnovsky fixative and processed afterwards using different microscopic techniques. Low temperature scanning electron microscopy (LTSEM) showed hyphae and encysted zoospores 24 h after inoculation. Root sections (10-15 µm) treated with calcofluor white were observed under epifluorescence microscopy and *P. cinnamomi* hyphae covering root surface and going progressively through cortex tissue were also observed. Roots sections (8-10 µm) stained with safranin-fast green and observed under light microscopy showed hyphae on the external root tissue 24 h after inoculation. Light microscopy was also used with 2 µm root sections stained with toluidine blue and allowed the detection of *P. cinnamomi* hyphae penetrating the parenchyma tissue. Finally, 80 nm root slices were obtained and examined through transmission electron microscopy (TEM). *P. cinnamomi* penetrated the cell walls forming haustorial-like structures and also grew through intercellular spaces.

+ Poster 14

Quick dissemination of *Phytophthora cinnamomi* threatens biodiversity in a World Heritage Site (Doñana Biological Reserve, SW Spain)

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Scattered big trees play a main role in maintaining biodiversity in savannah-like ecosystems. In this study we analyze the case of a remnant centenarian Cork oak (*Quercus suber* L.) population in the Doñana Biological Reserve (DBR, SW Spain), a Biosphere Reserve. Previous studies demonstrated that it was threatened by herbivorous and nesting wading birds proliferation [1]. Several sudden dieback events not related to any of previously reported causes lead us to investigate the potential role of pathogenic oomycetes.



Along four years of study period (2008-2011) tree rizosphere was increasingly being colonized by *Phytophthora cinnamomi*. We found that 2010 late winter/ early spring rainfall values exceeded all previous (32 yr) records and significantly extended the period with flooding/high soil moisture towards warmer months. These outstanding climatic conditions seem to have favoured the massive spread of this invasive water-dependent pathogen.

On the other hand, we found a significant correlation between the occurrence of *P. cinnamomi* in 2008/9 and tree crown health status in late 2010, suggesting a delay between pathogen arrival and the appearance of crown symptoms.

We analyzed future perspectives for pathogen spreading and discussed the feasibility of implementing currently available control measures in the context of a highly protected biodiversity reserve. We conclude that only individual treatments could allow for simultaneously save infected trees, prevent infections on healthy trees and avoid any chemical release to the environment.

[1] García, L.V., Ramo, C., Aponte, C. Moreno, A., Dominguez, M. T., Gómez-Aparicio, L., Redondo, R and Marañón T. Protected wading bird species threaten relict centennial cork oaks in a Mediterranean Biosphere Reserve: a conservation management conflict . *Biological Conservation* **144** 764–771 (2011).



SESSION 7

MANAGEMENT AND CONTROL



A chronicle of the impacts of sudden oak death in California: Fifteen years of invasion

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More than fifteen years after the first observations of sudden oak death, *Phytophthora ramorum* continues to spread in communities, parks, forests and nurseries in California. In the United States, well over \$100 million has been expended for regulation enforcement, research, management and education to combat this exotic, quarantine pathogen. Are these investments justified? This paper utilizes research findings, legislative and media activity, funding history and anecdotes to display the impacts of sudden oak death and chronicle the multitude of ways this microbe has damaged the environment. The pathogen has killed millions of tanoak, *Notholithocarpus densiflorus* and coast live oak, *Quercus agrifolia* over hundreds of square miles. Tree mortality is scattered over lands in a range of uses: wilderness, residential property, roadsides, parks, commercial forest and tribal gathering lands. However, overtime, sudden oak death-induced tree mortality fluctuates widely in extent and appearance. The economic value of losses from impaired ecological services and property damage are difficult to estimate. The social impacts are intangible and transient. As a result, the importance of sudden oak death is viewed differently by various interest groups. The reactions to sudden oak death reflect how Americans values trees and demonstrates the challenges of addressing tree pathogens.

Monitoring the effectiveness of *Phytophthora ramorum* eradication treatments in Oregon Tanoak Forests

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Phytophthora ramorum, the cause of sudden oak death, was first discovered in Oregon forests in July 2001. An aggressive eradication treatment program consisting of cutting and burning infected and exposed host plants, and where possible, injecting herbicide, was immediately put into place on all lands where it was found. To monitor the effectiveness of treatments we are revisiting treated sites and sampling soil and vegetation on fixed-area plots centered on stumps of infected trees. We established 145 plots in 2008-2009 and 143 plots in 2010. 109 of these plots were visited in both time periods.

Phytophthora ramorum was not recovered from soil or vegetation on 74 plots sampled in 2008-2009. Forty-seven plots yielded *P. ramorum* from soils only. The pathogen was present in soil and vegetation on 18 plots, and was recovered from vegetation only on six plots. In 2010, *P. ramorum* was not recovered from soil or vegetation on 90 of the plots sampled. Thirty-six plots yielded *P. ramorum* from soil only, on ten plots the pathogen was present in soil and vegetation, and on seven plots, *P. ramorum* was recovered from vegetation only. All positive vegetation samples were from tanoak in 2008-2009. Two *P. ramorum*-positive samples of Oregon myrtle were collected in 2010 samples along with infected tanoak sprouts.

Analysis continues on these data. Of particular interest is how different components of the treatment prescriptions and/or abundance and composition of post-treatment vegetation affect pathogen survival and disease development. These data are also being used to inform 2012 sampling.



Managing sudden oak death in Oregon forests, 2001-2011

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Sudden Oak Death, caused by *Phytophthora ramorum*, is lethal to tanoak (*Notholithocarpus densiflorus*) and threatens this species throughout its range in Oregon. The disease was first discovered in coastal southwest Oregon forests in July 2001. An interagency team attempted to eradicate the pathogen through a program of early detection surveys followed by destruction of infected and nearby host plants. Eradication treatments eliminated disease from many infested sites but the disease continued to spread slowly in a predominantly northward direction. During the 10-year period, the disease spread from the initial infestations southward 1.9 km, and northward and eastward 27.9 km and 7.6 km, respectively. We attribute continued spread of sudden oak death to the slow development of symptoms in infected trees which hinders early detection, and to delays in completing eradication treatments which allow disease spread from known infestations.

A marked increase in disease in 2010 and 2011 indicated that eradication costs on private lands would exceed available or expected funds. In early 2012 the Oregon State quarantine regulations were revised to reflect the financial reality of managing sudden oak death. The initial goal of complete eradication in Curry County forests is now considered unachievable. Our goal now is to slow further disease spread by: 1) early detection and rapid eradication of new infestations that are epidemiologically important; 2) reducing inoculum levels wherever practical through cost-share projects and best management practices, and; 3) improved education and outreach to prevent spread by humans. Lessons learned during the Oregon eradication effort are discussed.

Mitigating against dispersal of *Phytophthora* spp. on forest produce

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In the past two decades a disturbing number of serious new *Phytophthora* diseases have been recognised around the world. These have generally been the result of a new association between a species of *Phytophthora*, and a host that has not previously been encountered. Both described *Phytophthora* species, and those that were unrecognised prior to the emergence of the new disease, have been implicated. Often the origin of an incursion is not known, however the movement of tree products pose a potential risk for such introductions through the international trade of raw forest products. With increasing globalisation of trade there is concomitant concern that many more diseases caused by *Phytophthora* species will be discovered as these organisms are dispersed both nationally and internationally.

The potential for elimination of propagules of *Phytophthora* spp. from forest produce has been examined. Spores of *Phytophthora* species were applied in aqueous suspension to the bark on wood segments at densities of 350-550/cm², incubated at five temperatures likely to be encountered in transit. Material was tested for survival of the inoculum at successive intervals thereafter. The efficacy of the fumigants methyl bromide, phosphine and sulfuryl fluoride on the elimination of surface contamination was also tested.

The extent of possible natural contamination of forest produce remains to be determined but it appears



likely that *Phytophthora* spp. surface-contaminating products leaving the forest will survive for only a short period of time.

Survival and eradication of *Phytophthora cinnamomi* from black gravel graveyard sites in the *Eucalyptus marginata* (jarrah) forest

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Phytophthora cinnamomi is known to survive more than 50 years on impacted sites in the *Eucalyptus marginata* forest. One of the most severely impacted landscapes within this area are the 'black gravel' sites and persistence of the pathogen has made these areas extremely difficult to rehabilitate. Previous research has shown that *P. cinnamomi* is a poor competitive saprophyte so it was postulated that complete removal of the vegetation will kill the pathogen. Eradication experiments on black gravel sites investigated the length of time *P. cinnamomi* can survive in the soil without living plant tissue. Results encourage the view that the pathogen can be eliminated from infested sites as recoveries decreased significantly two years after removal of living plants. Annual and herbaceous perennials play an unexpectedly important role in the disease cycle and must be eliminated if eradication is to be successful.

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Investigations on control measures for *Phytophthora ramorum* and *Phytophthora kernoviae* in heritage gardens and parks

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In Britain, *Phytophthora ramorum* and, to a lesser extent, *Phytophthora kernoviae*, have caused die-back of invasive *Rhododendron ponticum* in woodlands, *Vaccinium myrtillus* (bilberry) in heathland and ornamental plants in nurseries and gardens. Plantations of *Larix kaempferi* (Japanese larch) have been severely affected by *P. ramorum* and have been cut down prematurely to reduce the risk of pathogen spread. The aim of this project ^[1] is to develop practical approaches for managing both pathogens in heritage gardens and parks.

Approaches being tested include:

- 1) Tree injection: The fungicides mandipropamid, mefenoxam and potassium phosphite were injected into *Magnolia* and forestry larch trees at infected locations in South West England. Leaves were assayed for residues and are being monitored for the presence of both pathogens.
- 2) Soil / leaf litter disinfestation: Products containing dazomet, mustard meal, *Trichoderma harzianum* T-22 or *Gliocladium catenulatum* J1446 have been incorporated into soil before re-planting. In other plots, mulches of woven ground-cover material, bark chips, wool waste mats, copper hydroxide treated mats and heat-treated wood shavings have been placed around rhododendron plants to prevent the splash-up of infested soil.
- 3) Fungicide applications: Based on detached leaf assays, several fungicides were selected for efficacy and tested on new plantings of rhododendron and pieris. Applications with products containing ametoctradin + dimethomorph, benthiavalicarb-isopropyl + mancozeb, fluopicolide + propamocarb



hydrochloride or metalaxyl-M + mancozeb were made in 2011 under conditions of natural infection by *P. ramorum* and *P. kernoviae*.

- 4) Susceptibility periods and susceptible hosts: Field observations and a desk study are being carried out to aid planting decisions.

All the experiments are currently being assessed and results will be presented.

[1] Key Research Findings from research commissioned from 1st April 2009 in support of the 5-year Defra "Phytophthora Disease Management Programme". Project Code PH0604.
www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/phytophthora/documents/prPkResearchJan12.pdf



SESSION 8

MANAGEMENT AND CONTROL IN AUSTRALIA



The state of *Phytophthora* science and management in natural ecosystems is Australasia

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Since the last IUFRO held in New Zealand 2010 there has been significant activity with regards to research and management of *Phytophthora* species in natural and peri-urban forest, woodland and heathland ecosystems in Australasia. For example, numerous new *Phytophthora* species have been described with a number of others in progress. There are numerous stable hybrid *Phytophthora* species being found and questions are being asked about their role in natural ecosystems. Nurseries continue to play a role in the dissemination of *Phytophthora* species. Containment and eradication of *P. cinnamomi* at a management level has occurred for the first time in a range of diverse plant communities and varying soil types in Western Australia. This clearly shows that eradication is a viable option, although good hygiene measures still remain extremely important in reducing spread into disease-free areas. The sequencing of the *P. cinnamomi* genome has been completed. Research is on-going on determining how phosphite induces defence mechanisms in plants at a molecular and biochemical level. The interaction between drought and *P. cinnamomi* is being studied, particularly with regards to projected warming and drying in the next few decades by all climate models for southern Australia. Community engagement and participation remains high, although on-going funding remains an issue and will be one of the challenges for the future. These activities clearly indicate that *Phytophthora* diseases still remain a key concern in the Australasian context. These and other activities will be addressed in more detail at the meeting in Spain.

A comparison between liquid phosphite injections and novel soluble phosphite and nutrient implants to control *Phytophthora cinnamomi* in *Banksia grandis* and *Eucalyptus marginata*

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Stem injections with phosphite liquid protect *B. grandis* and *E. marginata* from *P. cinnamomi* for at least four years [1]. However, stem injection of phosphite is labour intensive and requires training, specialised equipment, and the mixing of chemicals. The recent development of soluble phosphite implants which can be quickly inserted into stems, overcomes the need for training and the use of specialised equipment. Systemic nutrient implants and injections have been effectively used to correct nutrient deficiencies in ornamental and horticultural plants and can help increase tree vigour to pests and pathogen attack. However, soluble implants of phosphite and nutrients have never been trialled for the control of *Phytophthora*. This study aimed to determine if liquid phosphite, soluble implants of phosphite alone, or combinations of macro and micro nutrients within implants inserted into the trunks of the trees could control lesion development caused by *P. cinnamomi*. In *B. grandis* and *E. marginata*, phosphite liquid and soluble phosphite implants significantly reduced lesion length compared to the control and application of nutrient implants. In *B. grandis* and *E. marginata*, nutrient implants reduced significantly the average lesion length compared to the control. Results show that both phosphite liquid and implants are effective at controlling lesion extension in *B. grandis* and *E. marginata*, caused by *P. cinnamomi*. Stem treatment with soluble phosphite implants will facilitate the rapid treatment of trees, and control of *P. cinnamomi*, in diseased areas. In addition, the uptake is passive, there is likely less damage to internal stem tissues, and less risk from phytotoxicity due to slow release of the phosphite



compared to the liquid treatments.

[1] Shearer, B. L., Fairman, R. G. and Grant, M. J. Effective concentration of phosphite in controlling *Phytophthora cinnamomi* following stem injection of *Banksia species* and *Eucalyptus marginata*. *Forest Pathology*, **36** 119-135 (2006).

Community involvement in Phytophthora dieback management - looking back and forward

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The south west corner of Western Australia is internationally recognised as a biodiversity hot spot. The federal and state governments recognise *Phytophthora cinnamomi* as one of the biggest threats to this biodiversity. The community has been playing an important role in the management of *Phytophthora* dieback since 1993. By the early 1990s the State Government environment department and large mining companies were routinely implementing prescriptions to minimise the spread and impact of *P. cinnamomi*. These prescriptions were based on nearly 30 years of research that had discovered that this introduced pathogen spreads during soil movement and in the surface runoff of water from infested sites. The successful use of phosphite to protect susceptible plants from *P. cinnamomi* had also been demonstrated. Meanwhile, no management of *Phytophthora* dieback was taking place in natural ecosystems being managed by other state government departments, local governments and private landowners. The spread of disease in these ecosystems was termed 'inadvertent' - 'inadvertent' because the land managers did not know about the disease or how to minimise its spread and impact. It was the community that mobilised to address this knowledge gap. In 1993 and 1994 the education of two local governments was completed. In 1995 the community-based Dieback Working Group formed and the program to facilitate the adoption of *Phytophthora* management policies and prescriptions commenced in earnest. Nineteen years later no management plan for a local government bushland reserve would be submitted without *Phytophthora* dieback management being addressed. Project Dieback was launched in 2004 to ensure integrated management of threats to biodiversity at the regional scale regardless of land tenure and to address gaps in strategic, regional, dieback planning. Community has guided and embarked on protecting native vegetation from *Phytophthora* infestation in a number of regional priority areas. The talk will focus on the list of successes, the reasons for the successes but also highlight the current gaps that still need to be plugged – our work is not done!



Successful containment and eradication of *Phytophthora cinnamomi* at a management level from diverse natural ecosystems in Western Australia

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Here we demonstrate that management scale containment and eradication can be achieved for *Phytophthora* dieback infestations in three different native plant communities in Western Australia. The communities include: (1) a Kwongan vegetation type on a soil varying from sandy to exposed rocky subsurface to a clay above a rock subsurface, (2) a Proteaceous heathland on a deep sand profile, and (3) a Kwongan Banksia woodland on a deep sand over a sandy loam-clay. All of these communities are highly impacted by *P. cinnamomi* resulting in substantial loss of biodiversity assets. The successful approach taken involved the following tasks: risk assessment of the project goals and proposed techniques, implementation of hygiene plans, extensive and intensive soil and plant sampling and *in situ* baiting to accurately map the occurrence of the pathogen, detailed hydrological characterisation using remote sensing techniques, 2D hydraulic modelling, development of hydrological engineering options, catchment modelling, installation of fences to reduce animal vectoring, herbicide applications to remove living host support for the pathogen, phosphite foliar sprays, fumigation with metham sodium and on-going monitoring of the sites to demonstrate the success of the approach taken. Prevention of further spread through these high priority natural ecosystems is now of high priority. This project has involved partnerships between government and non-government agencies, industry, researchers and community groups. These partnerships have included the construction of hygiene infrastructure around priority National Parks and the engagement of key stakeholders in the management of Phytophthora Dieback. The approach described has huge potential for the eradication and containment of other soil-borne *Phytophthora* species around the world.

Dunstan WA, Rudman T, Shearer BL, Moore NA, Paap T, Calver MC, Dell B, Hardy GESJ Containment and spot eradication of a highly destructive, invasive plant pathogen (*Phytophthora cinnamomi*) in natural ecosystems. *Biological Invasions*, **12** 913-925 (2010).



POSTERS



6th IUFRO Working Party 7.02.09
“*Phytophthora* in Forests and Natural Ecosystems”
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Assessment of the presence of oomycetes in forest nurseries in Eastern Spain

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Sanitary quality of seedlings is one of the several factors involved in the survival of plants in the field. An extensive survey was conducted in forest nurseries throughout the eastern Iberian Peninsula in order to assess the presence of oomycetes and plant pathogenic fungi in plant material to be used in reforestation programs. Plants showing disease symptoms such as dieback, wilting, chlorosis, leaf spots, discoloration of needles, aborted buds, defoliation and / or stunting were collected as well as the substrate contained in their pots. Asymptomatic plants were also randomly sampled. Isolation from plant material was performed onto CMA-PARPBH and PDAS. Apple baiting method was used to recover oomycetes from soil samples. After incubation at 25°C in the dark, hyphal tips were transferred to PDA and V8-Agar media for further studies and conserved on OA medium. *Phytophthora* and *Pythium* isolates were identified based on morphological, physiological and molecular information among the following hosts: *Pinus halepensis*, *P. nigra*, *P. pinea*, *P. pinaster*, *P. sylvestris*, *Arbutus unedo*, *Ceratonia siliqua*, *Fraxinus ornus*, *Quercus ilex* subs. *Ballota*, *Sorbus domestica* and the shrub species *Cistus albidus*, *Myrtus communis*, *Pistacia lentiscus*, *Viburnum lantana*, *Viburnum tinus*. The oomycetes *Phytophthora cactorum*, *P. citrophthora*, *P. cryptogea*, *P. nicotianae*, *P. plurivora*, and several isolates of *Pythium* were identified from affected roots and substrate. At the moment no link has been found between the *Phytophthora* species isolated in these surveys with those detected in the natural ecosystems studied in Eastern Spain.

Effect of three control treatments on the survival of *Quercus ilex* seedlings infected with *Phytophthora cinnamomi*

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Phytophthora cinnamomi is an invasive root pathogen that causes decline and mortality in main evergreen oaks species in Spain. Several fungicide treatments have been tested both in vitro [1] and in vivo [2] against *P. cinnamomi* and chemical treatment could play a major role in future control strategies for *Phytophthora* root rot.

Holm oak (*Quercus ilex*) seedlings 15 month-old cultivated in greenhouse were treated with potassium phosphonate applied to the stem (300 µL 2.5%/plant+ surfactant), soluble silicon soil drench (75 ml 2% SiO₂/plant) and gypsum amendment (5% p/p) before being inoculated with a chlamydospore suspension of a *P. cinnamomi* isolate with proven virulence. Potting media were kept moist for optimal infection and eventually flooded with tap water during 5 days. Mortality was registered during 12 weeks. Mortality was modeled with generalized linear model and treatment effects were compared by deviance analysis. No mortality occurred before the plants were flooded. Inoculation with *P. cinnamomi* reduced survival of plants from 95% to 78%, however none of the treatments used was able to reduce the mortality and survival time in the inoculated plants.



- [1] Bekker, T.F., Kaiser, C., Van Der Merwe, R. and Labugchagne, N. In-vitro inhibition of mycelial growth of several phytopathogenic fungi by soluble silicon. *S.A. J. Plant Soil*, **26** 169-172 (2006).
- [2] Serrano M. S., De Vita, P., Fernández-Rebollo, P. and Sánchez, M. E. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology*, **132** 271-279 (2012).

Poster 3

Pythiaceae and fungal species isolated from coniferous and deciduous seedlings in some Turkish nurseries

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Turkish private and state owned forest nurseries have been responsible for the supply of increasing numbers of seedlings for afforestation. The most important phytopathological problems in forest nurseries are mainly caused by root rot fungi and Pythiaceae species like *Phytophthora*, *Phytopythium* and *Pythium*, which bring about severe economical losses in seedling production [1].

In this study, forest nurseries of Denizli, İzmir, Adapazarı, Bursa and Muğla Provinces were investigated for the presence of root rot fungi and *Phytophthora*, *Phytopythium* and *Pythium* species. For this purpose potted and bare rooted seedlings showing collar rot, chlorosis, wilting and dieback symptoms and also soils of coniferous and deciduous trees species were sampled. Isolations from symptomatic roots were performed on selective PDA medium. Soil samples were baited with carnation, and young leaves of rhododendron and cork oak. Infected leaves were placed onto petri dishes containing selective PARPNH agar. Identification of isolates was performed both based on colony patterns, growth rates, and morphological features and molecular methods [2]. Internal transcribed spacer sequences of ribosomal DNA, 820 bp long, were amplified using the ITS1 and ITS4 primer pair. *Abies bornmülleriana*, *Quercus virginiana*, *Thuja occidentalis*, *Pinus sylvestris*, *Quercus suber*, *Platanus orientalis*, *Buxus sempervirens*, *Laurus nobilis*, *Castanea sativa* seedlings were found to be infected by root rot fungi and Pythiaceae species. *Fusarium oxysporum*, *F. moniliforme*, *Fusarium* spp. *Rhizoctonia solani* Kühn., *Alternaria* spp. *Cylindrocarpum destructans* were the main fungal species commonly isolated from seedlings. Beside these fungal species *Pythium ultimum*, *Pythium irregulare*, *Phytopythium vexans*, *Phytopythium litorale*, *Phytophthora cactorum*, *P. citricola* and *P. plurivora* were identified so far. Morphological identifications and ITS region sequence comparisons of more Pythiaceae species are still going on.

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[2] Lilja, A., Kokkola, M., Hantula, J., Parikka, P. *Phytophthora* spp. a new threat to tree seedlings and trees. In: Solheim, H. & Hietala, A.M. (eds.). Forest pathology research in the Nordic and Baltic countries 2005. Proceedings from the SNS meeting in Forest Pathology at Skogbrukets Kursinstitutt, Biri, Norway, 28-31 August 2005. Aktuelt fra Skogforskningen 1/06: 48-51. (2006).

Poster 4

***Phytophthora bilorbang* prov. nom., a new species associated with declining *Rubus anglocandicans* (blackberry) in Western Australia.** S. Aghighi, G. Hardy, J. K. Scott, T. Burgess

See Session 5



Nature of *Phytophthora* inoculum in flowing surface waters. K. Aram, D.M. Rizzo

See Session 3

Characterisation of the two informally designated ITS Clade 6 taxa *Phytophthora* taxon Forestsoil and *P. sp. hungarica*

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Phytophthora ITS Clade 6 contains numerous informally designated taxa and recently described new species. We report here on the characterisation of the two informally designated taxa *P. taxon Forestsoil* and *P. sp. hungarica*. The first culture of *P. taxon Forestsoil* was collected from soil of an oak-*Carpinus* forest in 1998 and partially characterised later [1]. Subsequently, a few isolates from alder soil with identical ITS sequences to *P. t. Forestsoil* were reported as *P. sp. sylvatica* and *P. sp. H-6/02* at Genbank in 2007. At the same time other isolates from alder soil, differing from *P. t. Forestsoil* and its synonyms in their colony pattern on carrot agar (CA) and at the same 4 positions in their ITS, were named *P. sp. hungarica*, *P. sp. H-7/02* and *P. sp. H-8/02*. Additional ‘hungarica’ isolates were then collected in Alaska [2], also from alder soils or nearby waterways. In our detailed study, the only consistent morphological difference between ‘Forestsoil’ and ‘hungarica’ was still the culture pattern on CA. Both taxa showed similar growth rates and cardinal temperatures, produced similar sporangia, were homothallic with mostly paragynous antheridia and aplerotic oospores often with several ooplasts. Concatenated analyses using 4 nuclear and 2 mitochondrial gene sequences were in concordance with the morphological and physiological features supporting the grouping of ‘Forestsoil’ and ‘hungarica’ isolates into 2 very closely related but distinct clades. These results might help to unravel the taxonomic and phylogenetic position of these informally designated taxa.

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[2] Adams, G.C., Catal, M. and Trummer, L. Distribution and severity of alder *Phytophthora* in Alaska. In: Frankel, S.J., Kliejunas, J.T. and Palmieri, K.M. (tech. coords.) Proceedings of the Sudden Oak Death Fourth Science Symposium. Gen. Tech. Rep. PSW-GTR-229. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 29–49 (2010).



Population fluctuation of *Phytophthora* spp in streams and their possible role as early colonizers.

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The population fluctuation of *Phytophthora* was quantified for a one-year period in two streams. The Paint Branch creek (PB) and Comcast Center creek (CC), part of the Anacostia watershed in Maryland, were sampled. The CC creek was considerably smaller and is a tributary of PB. Streams differed in both their size and the amount of water available. Every week for two days (Monday and Wednesday) 1 L water sample was collected twice at 8 AM and 2 PM. Of the 1 L sample, two or three 200 ml subsamples were filtered through a 3 µm pore size 9 cm diameter nitrocellulose Millipore membrane filter and placed on clarified V8 juice based PARPNH growth media selective for isolation of *Phytophthora* spp. After two days of incubation in darkness at room temperature, colonies were counted. Streams differed significantly in *Phytophthora* colony numbers. The smaller CC stream had almost twice as many colonies compared to PB stream. However, there was no significant difference between the samples that were collected during the morning (8am) or afternoon (2pm). A significant decrease of population of *Phytophthora* was found with increasing temperatures in both streams. Sampling month played a significant role in *Phytophthora* colony numbers. In both streams June, October and November had the greatest number of colony counts. When live and dead leaf baits (leaves were oven dried for one week) of various plants were deployed at CC stream. Overall, more *Phytophthora* colonies were isolated from live compared to dead leaves. However, with red maple (*Acer rubrum*) and red oak (*Quercus rubra*), dead and live leaf baits were equally colonized by *Phytophthora* spp.

Phytophthora species associated with disease in peri-urban woodland and forest ecosystems

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Perth, the capital city of Western Australia, is situated on a river with numerous bays. The peri-urban environment extends along the coast for 100 km north and south of the city. Within the region is a fragmented landscape of suburbs, parks and remnant woodlands. The *Eucalyptus gomphocephala* woodland south of Perth is classified within the peri-urban environment. Numerous *Phytophthora* species have been isolated from dead and dying endemic trees. The most frequently isolated species is *P. multivora* (65%), followed by *P. aff. arenaria* (21%); *P. palmivora*, *P. syringae*, *P. inundata*, *P. aff. humicola*, *P. nicotianae* and *P. sp. ohioensis* have also been isolated, although rarely. *P. multivora* and *P. aff. arenaria* have both been isolated from dying *E. marginata* (jarrah), *E. gomphocephala* (tuart), *Corymbia calophylla* (marri), *C. ficifolia* (red flowering gum) and *Agonis flexuosa* (WA peppermint). While *P. multivora* is commonly encountered in less impacted ecosystems, the other species found in the peri-urban environment (*P. inundata* and *P. nicotianae*) are rarely or never isolated. In the riparian ecosystem, *P. aff. humicola* has been isolated from dying *Casuarina obesa*. The knowledge about the impact of these species on our remnant trees is lacking. Further research is required on the origin, pathogenicity and control of these species to deliver effective management strategies.



A species concept for *Phytophthora* taxon *Agathis* (PTA) — causal agent of root and collar rot of *Agathis australis* in New Zealand. S. E. Bellgard, B. S. Weira, P. R. Johnston, D. Park, D. J. Than, N. Anand, S. R. Pennycook

See Session 5

Diversity of oomycetes detected in the laurel forest in Tenerife (Canary Islands)

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The laurel forest (laurisilva) is a unique type of vegetation found on the Canary Islands, Madeira and the Azores. It is found in areas with high humidity and relatively stable and mild temperatures. This type of forest is a unique ecosystem, centre of plant diversity, and of great importance for conservation. The objective of this study was to investigate the presence of oomycetes that could be threatening the health of the laurisilva vegetation in the Anaga Rural Park (14419 ha) at the north-eastern end of the Island of Tenerife. A survey was carried out during autumn 2011 and samples of roots and soil were collected from symptomatic and asymptomatic trees and shrubs (*Ocotea foetens*, *Arbutus canariensis*, *Viburnum rigidum* and *Persea indica*) in 22 different locations in the Anaga Rural Park. Direct isolation from roots was performed onto PARPH and avocado leaves were used as baits for soil isolation. Sixty eight oomycete isolates were obtained and grouped on the basis of the morphology of their vegetative and reproductive structures. The ITS region of the ribosomal DNA of isolates from each group was amplified and sequenced with the primers ITS4 and ITS6. Sequences obtained were compared with sequences in GenBank. *Phytophthora multivora* was the only *Phytophthora* sp. detected in this preliminary survey. Five different *Pythium* spp. were detected: *P. diclinum*, *P. heterotallicum*, *P. litorale*, *P. mamillatum*, *P. mercuriale* and *P. vexans*. Three *Pythium* spp. could not be identified on the basis of their morphology and sequences. This is the first detection of these oomycetes in the laurel forest in the Canary Islands. Further work to study the pathogenicity of these oomycetes is needed in order to evaluate the risk that they might pose to the laurel forest.

Diversity and distribution of *Phytophthora* species in association with water quality and the health of trees in fragmented riparian ecosystems

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The riparian zone in western Australia is dominated by *Eucalyptus rudis* in a similar manner to *Alnus* spp. in Europe. For the last 20 years the health of these trees have been declining. This is attributed to an endemic leaf sucking Psyllid, however contributing factors could be an increase in the salinity of the waterways or the presence of a root pathogen, both of which would reduce the health of the trees. We sampled 25 sites along different rivers and streams in the southwest of Western Australia. At each site we recorded tree health, determined water quality and filtered water for the isolation of pythiaceus oomycetes. There was considerable variation in water quality (pH and salinity) and the health of the



adjacent *E. rudus*, however the poor health was not related to low water quality. There was also considerable variation in the number of colony forming units (from 1.33 to 90 L⁻¹), the proportion of *Phytophthora* compared to *Pythium* isolates (from 0-100%) and the species biodiversity. In general, far more isolates were obtained from low quality water, except for when the pH was greater than 8.5. Water quality did not effect the proportion of *Phytophthora* isolates. *Phytophthora* species isolated included *P. thermophila*, *P. fluvialis*, *P. amnicola* and hybrids between these species. Additionally, numerous isolates of *P. taxon salix* soil were obtained. Remnant sites closer to the urban area contained predominantly *P. thermophila* while *P. taxon salix* soil predominated on the more southerly sites from remnants within agricultural zones. The link between tree health, water quality and associated pythiaceus populations was not established in this study.

Poster 12

Spatial patterns of holm and cork oak decline in Extremadura, Spain. E. Cardillo, A. Acedo, C. Perez

[See Session 5](#)

Poster 13

Next Generation Sequencing shows *Phytophthora* species diversity in soil samples of Macaronesian laurel forests from the Canary Islands

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Laurel forests (also called laurisilva) are exclusive of the Macaronesian biogeographical region, which comprises three archipelagos: Madeira, Azores and Canary Islands. The main vegetation of the laurisilva in the Canary Islands is dominated by tree and shrub species with laurel-shaped leaves, like *Ocotea foetens*, *Arbutus canariensis*, *Viburnum rigidum*, *Myrica faya* or *Persea indica*, which are developed under high humid conditions. The geographical isolation of the volcanic islands has stimulated the emergence of numerous endemic organisms. However, little is known about the biodiversity of soil microorganisms. Soil DNA was extracted from ten samples from different locations in the Anaga Rural Park (Tenerife). ITS1 amplicons were obtained through a nested PCR using *Phytophthora*-specific primers 18Ph2F and 5.8S-1R [1] in PCR1 and fusion primers based on ITS6 in PCR2, and pyrosequenced on a Roche Junior GS platform (454 Life Sciences). Six of the samples produced amplification signal. The results obtained in this work will bring light into the diversity of *Phytophthora* spp. in this unique ecosystem.

[1] Scibetta, S., Schena, L., Chimento, A., Cacciola, S. O. and Cooke, D. E. L. A molecular method to assess *Phytophthora* diversity in environmental samples. *J. Microbiol. Meth.*, **88** 356-360 (2012).



Quick dissemination of *Phytophthora cinnamomi* threatens biodiversity in a World Heritage Site (Doñana Biological Reserve, SW Spain). P. De Vita, L. V. García, C. Ramo, M. S. Serrano, C. Aponte, M. E. Sánchez

See Session 6

Influence of bird faeces in the behaviour of the root rot of *Quercus suber* caused by *Phytophthora cinnamomi* at Doñana Biological Reserve (SW Spain). P. De Vita, M. S. Serrano, L. V. García, C. Ramo, M. E. Sánchez

See Session 3

Airborne infections of *Phytophthora citrophthora* on citrus in Sicily

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Citrus species native to the far-east of Asia have been introduced into the Mediterranean area as cultivated plants since time immemorial and are grown on a large scale for their fruits, and presently they characterize the Sicilian landscape. Two exceptional climatic events, a hail storm on 7th March, 2012 and on 9th March hurricane Athos, with blasts of wind with a speed of 80-100 km/h, hit the southern-east coastal area of Sicily. As a consequence, severe fruit brown rot infections were observed on the higher part of the canopy up to 3.4 m above soil level in citrus groves. Fruit symptoms were associated with leaf blight and severe defoliation of the trees. In the past, these kinds of aerial infections of citrus brown rot in Sicily had been attributed to *Phytophthora hybernalis* on the basis of symptomatic diagnosis. However, a survey of citrus orchards affected by these exceptional climatic events using molecular diagnostic methods demonstrated that the causal agent of this epidemic burst of brown rot was actually *P. citrophthora* (Smith & Smith) Leonian. This species is endemic in citrus orchards in Sicily and is the major causal agent of trunk gummosis, root rot and occasional epidemic outbreaks of fruit brown rot of citrus occurring from late autumn to early spring, which is the rainy period in the Mediterranean climate. *P. citrophthora* is typically a soil-borne pathogen and usually infects fruits of the lower part of the canopy near the soil up to 1 m.

ITS based identification and phylogeny of *Phytophthora* spp. detected in UK gardens

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Many studies of *Phytophthora* species have been conducted in commercial settings, such as nurseries, and wild habitats, often forgetting our own back gardens. Gardens are unique environments with plants being a mixture of natives and non-natives from around the world. The current work will detect and



identify the *Phytophthora* species present in these microhabitats. Additionally, this artificial mixture of plant species enables investigation into the potential host range of *Phytophthora* species on both native and non-native plants. Environmental samples, from UK gardens, were surveyed during 2006 to 2009 for the presence of *Phytophthora* species, comparing apple baiting with a nested PCR protocol based on the ITS region [1]. The ITS region has become increasingly used for identification of *Phytophthora* species via direct sequencing, development of arrays, real-time PCR and used in many phylogenetic studies. The ITS region from the recovered isolates was sequenced and phylogenetic analysis was undertaken of the amplicons from both cultures and nested PCR. Phylogenetic analysis, incorporating host and morphological data, will be presented. Through baiting the species *P. cactorum*, *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. gonapodyides*, *P. plurivora* and *P. niederhauserii* were identified. With the nested PCR a wider range of *Phytophthora* species were detected, *P. alni*, *P. austrocedri*, *P. cambivora*, *P. hibernalis*, *P. megasperma*, *P. porri*, *P. quercina* and *P. syringae* were additionally identified. This will be the first study of UK gardens presenting species identification and phylogenetic analysis.

[1] Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. and Brasier, C. M. A molecular phylogeny of *Phytophthora* and related Oomycetes. *Fungal Genetics and Biology* **30** 17-32 (2000).

Poster 18

Pathogenicity of *Phytophthora* species on *Liquidambar orientalis* and *Castanea sativa* seedlings

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Liquidambar orientalis is one of the endemic tree species which is native to the eastern Mediterranean Region and *Castanea sativa* is the economically important tree species widely growing from the Black sea coast to Marmara Region and western Anatolia. This study was performed to test the pathogenicity of *Phytophthora* and *Phytophythium* isolates obtained from soil samples of both tree species [2]. For this purpose, *Phytophthora cactorum*, *P. plurivora*, *P. citricola*, *Phytophythium vexans*, *Phytophythium citrinum* and *Phytophythium litorale* isolates were inoculated to *Quercus suber* and *Quercus robur* seedlings using two different methods [1]. In the first method, isolates were grown in fine vermiculite and millet seeds moistened with carrot broth and the inocula were placed inside glass tubes near the seedlings. In the second inoculation method, *Phytophthora* and *Phytophythium* isolates were grown on carrot agar and were inoculated under the bark of represented seedlings. The inoculated seedlings were incubated at 20°C in a growth chamber for 3 months [1]. After the incubation period mortality, infection incidence, and lesion length under the bark were examined. Random reisolations were made using selective PARPNH-agar media to confirm *Phytophthora* and *Phytophythium* species from the lesions. As a result of the first test, mortality observed on both *Quercus* species inoculated with *Phytophythium* isolates was lower than that for *Phytophthora* isolates. *P. cactorum* was the most aggressive species within all *Phytophthora* species tested. In the second pathogenicity trial, average mortality and infection incidence on both hosts were similarly higher when the seedlings were inoculated with *P. cactorum*, *P. citricola* and *P. plurivora*, respectively. Eventhough the *Phytophythium* species were found to be less aggressive the results indicated that both *Phytophthora* and *Phytophythium* isolates were pathogenic on *Quercus* species tested.

[1] Jung, T., Blaschke, H., and Neumann, P. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands *European Journal of Forest Pathology*, **26** 253-272 (1996).

[2] Doğmuş-Lehtijärvi, H. T., Aday, A. G., and Jung, T. *Phytophthora* species from declining *Liquidambar orientalis* and *Castanea sativa* trees in south eastern part of Turkey. COST Action Meeting 21-22 November, Budapest, 32pp. (2011).



***Phytophthora ipomoeae* causing blight on *Ipomoeae orizabensis* in Michoacan, Mexico**

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Several wild species of *Ipomoea* grow in Michoacan, Mexico forests. *Ipomoea orizabensis* plants with blighted leaves and petioles were observed in a pine-oak forest located in Charo, Michoacan. Diseased leaves were placed in a humidity chamber to induce sporulation. Isolates were obtained on rye agar selective medium, transferring sporangia from sporulating lesions with a piece of agar to rye agar selective medium. A *Phytophthora* sp. was consistently isolated from blighted leaves. Species identification was based on sporangial and gametangial characteristics of three cultures grown on rye agar. Sporangia production was achieved placing mycelial discs in sterile soil extract. Sporangia were mainly ellipsoid but occasionally ovoid, semipapillated, and deciduous with a short pedicel. The isolates were homothallic with smooth walled and aplerotic oospores. Genotypic analysis for the allozymes Peptidase and Glucose 6- phosphate isomerase indicated that the isolates belonged to one genotype 96/96 (*Pep*) and 108/108 (*Gpi*). Morphological characteristics correspond to the species *P. ipomoeae* Flier & Grünwald. This species has been reported causing blight on *Ipomoeae* only in central Mexico. To confirm the identity of the pathogen, sequences of the internal transcribed spacers (ITS) were obtained from two isolates. The ITS sequences that were obtained shared 100% similarity with strains of *P. ipomoeae* from central Mexico. Pathogenicity tests are underway.

Research driving management and policy-making on Sudden Oak Death in California

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We briefly present results from different studies that are justifying specific disease management options and policies to curtail the spread of Sudden Oak Death in California.

- 1)- Yearly treatments with the phosphonate Agrifos has reduced mortality rate of tanoaks threefold in approximately 1000 studied trees from 32 study plots across the State
- 2)- Selective removal of bay laurels 10 m around oaks has significantly reduced, but not eliminated, the number of instances in which oaks are subject to inoculum levels sufficient to cause their infection. Removal of bays to 20 m, however, reduces that number of instances to zero
- 3)- Cuts on large branches and trunks are ten times more likely to become infected than unwounded trees right after the pruning, however after four months, pruned and un-pruned large branches are comparable in susceptibility
- 4)- Removal of all organic matter, soil, and plant debris until tools are visually clean reduces infectivity of tools to zero, without the need for chemical treatments
- 5) – Composting will kill the pathogen, but some composts can be infected once they become old, if exposed to high inoculum levels



6)- Population genetics analyses show that; a)- accidental introductions of the pathogen from infected nursery plants to the landscape were occurring as recently as 2006 in multiple locations, and b)- large wild infestations are the most likely sources of further infestations in California

The implications of these findings for disease management and policy-making will be discussed.

Poster 21

***Phytophthora acerina*, a new species from the *P. citricola* complex causing aerial cankers on *Acer pseudoplatanus* in Italy.** B. Ginetti, S. Moricca, A. Ragazzi, T. Jung

See Session 5

Poster 22

Reducing the spread of *Phytophthora ramorum* on the Redwood Nature Trail, Curry County Oregon: A case study

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In August 2009, a tanoak adjacent to a popular hiking trail was confirmed infected by *Phytophthora ramorum*. The trail was immediately closed to the public. An eradication treatment consisting of injected herbicide and cutting, piling, and burning tanoaks and other selected hosts in a 100m radius around the infected tanoak was completed by December 2009.

Close to 490 m of trail lies within or on the boundary of the treatment area while approximately 60m of trail passes through the infested zone. To limit the number of *P. ramorum* spores in soil and the potential for spore splash dispersal, a 4-inch thick layer of *Thuja plicata* heartwood chips was placed on the trail in July 2010. The trail was then reopened to public use.

Soil samples were collected at 11 locations on the trail four times prior to chip treatment and three times after chip treatment. *Phytophthora ramorum* was recovered from at least one of the 11 samples on all sampling occasions except in July 2010 and June 2011. The number of *P. ramorum*-positive soil samples was 2/11, 5/11, and 6/11 before-chip treatment and 1/11 and 1/11 samples after-chip treatment. All *P. ramorum*-positive samples were found within approximately 8m of the infected tree.

Phytophthora ramorum's presence in trail soil appears to have been reduced in the year after chip treatment. Recently, additional *P. ramorum* infections have been detected near the trail and due to use, chip depth has also been greatly reduced. Additional treatments will be done and monitoring will continue.

Poster 23

Spatial patterns of *Phytophthora cinnamomi* in declining Mediterranean forests: implications for tree species regeneration. L. Gómez-Aparicio, B. Ibáñez, M. S. Serrano, P. De Vita, J. M. Ávila, I. M. Pérez-Ramos, L. V. García, M. E. Sánchez, T. Marañón

See Session 3



Host and habitat index for *Phytophthora* species in Oregon forests (32 species!)

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Phytophthora species are abundant in streams in healthy forests and widespread in forest soils causing cryptic diseases, in addition to their more traditional roles as aggressive pathogens. We compiled existing Oregon records from available sources of reliably identified *Phytophthora* species from forests and forest trees and summarized the results by host and habitat (Forest Phytophthoras, <http://www.forestphytophthoras.org/>). Details of documented isolates including locations, available cultures, Genbank acquisition numbers, and citations are in the accompanying interactive database.

Thirty two *Phytophthora* species have been identified associated with 25 host species from Oregon forests or forest trees. This total includes 19 species recovered from forest streams and 19 from forest soils, generally in the absence of noticeable disease on associated vegetation. A total of 29 *Phytophthora* species were identified from the various environments in forests. Fourteen species came from trees or forest shrubs growing in cultivated and urban environments. Only three species were unique to the latter, however, including *P. ilicis*, from cultivated holly (*Ilex*), and *P. sansomeana* and *P. taxon ceanothus* from forest nurseries. Three species, *P. gonapodyides*, *P. taxon oaksoil*, and *P. taxon salixsoil* were recovered from streams in all surveyed counties. The most widespread species causing root disease or bole cankers of trees was *P. lateralis* on Port-Orford-cedar in landscape plantings throughout the state as well as on forest trees in its limited native range. *P. cambivora* and *P. cinnamomi* were widespread but uncommon on a number of forest trees.

Characterization of *Phytophthora alni* isolates from *Alnus glutinosa* in Castilla y León, Spain

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Extensive dieback and mortality of alder (*Alnus* spp.) reported from several European countries has also been observed in Spain in 2009. Surveys were conducted to collect information on disease symptoms and possible causes of decline of *Alnus glutinosa* growing on river banks of Castilla y León, Spain, in 2010 and 2011. Isolates were obtained from necrotic bark at collar and lower stems of the diseased alders. Morphological and molecular characteristics of the selected isolates together with their physiology were studied. The isolates were homothallic and produced oogonia on V8 juice agar (V8A) with single or two-celled amphigynous and paragynous antheridia having smooth to ornamented walls and bullate protuberances with a large quantity of aborted oospores. Long sporangiophores were found bearing terminal non-papillate, ellipsoid to ovoid sporangia. Colony growth patterns developed on carrot agar (CA) and V8 juice agar showed irregular to uniform radial growth having appressed to slightly woolly aerial overgrowth. Colony growth rates to different temperature, pH and osmotic potential varied as cultured on several growth media. ITS DNA region was sequenced, and compared with GenBank showing identity with *P. alni*, and confirming the morphological and physiological identification of the pathogen isolated. Complementary molecular studies are undergoing in order to identify the pathogen at subspecies level.



Persistence of *Phytophthora ramorum* on infested larch sites

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Phytophthora ramorum emerged in the 1990s almost simultaneously in both Europe and North America. It was first reported in the UK in 2002, but tree infections were comparatively rare until 2009. Then, a completely unanticipated change occurred with *P. ramorum* transferring to commercially grown larch causing a landscape-scale epidemic. Larch is both a foliar host and a canker host to *P. ramorum*. A single infected needle can generate hundreds, even thousands of sporangia, and chlamydospores are also produced. As the needles fall, the litter layer under infected trees becomes a reservoir of *P. ramorum* inoculum available to infect new plantings.

This study analysed the persistence of *P. ramorum* on a site in south west England, where stands of Japanese larch (*Larix kaempferi*) and European larch (*L. decidua*) showed disease symptoms. All the trees were felled, then each stand was surveyed systematically and both needle-litter and soil samples removed and baited. A total of 180 samples were taken from the Japanese larch stand and 132 from the European larch stand. One year later, the survey was repeated on the cleared Japanese larch stand. Two clear results emerged: (1) Much higher levels of *P. ramorum* were detected under the felled Japanese larch compared with the European larch (67% and 2% respectively); (2) A year after felling, levels of *P. ramorum* had reduced moderately in the felled Japanese larch stand. The findings support field observations that disease development on European larch is less than on Japanese. Also needles of Japanese larch usually support the highest levels of sporulation, consistent with the finding that *P. ramorum* was much more prevalent in fallen needles under this host species.

Comparative sporulation of *Phytophthora ramorum* on larch, rhododendron and bay laurel

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In Britain, *Phytophthora ramorum* has been recorded infecting plants since 2003, but tree infections were comparatively rare until 2009 when the pathogen was found killing commercially grown Japanese larch (*Larix kaempferi*) and causing a landscape-scale epidemic in south west England. This larch species is both a foliar host and a canker host to *P. ramorum* and initial studies showed that a single infected needle of Japanese larch could generate hundreds, even thousands of sporangia, suggesting this species has considerable potential to drive epidemics. However, the sporulation capability of other larch species grown in Britain, such as European (*L. decidua*) and hybrid larch (*L. x eurolepis*), is unknown.

To evaluate the spore producing potential of foliage of the three species of British-grown larch and compare with other known sporulating hosts (eg *Umbellularia californica* and *Rhododendron ponticum*), laboratory tests were carried out using shoots of Japanese larch, hybrid larch and European larch challenged with zoospore suspensions of *P. ramorum* (EU1 lineage). These tests were carried out at different times of year and have shown that sporulation potential varies with larch species, pathogen genotype and also with the age of the foliage. Japanese larch generally supported the highest levels of sporulation, even exceeding that on *U. californica*. Sporulation on larch needles can also occur in the absence of any symptoms particularly early in the season and in the field; symptoms on infected needles only become visible towards the end of the season just before they are shed.



***Phytophthora pini* found on thuja in Norway**

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In October 2011, we received a thuja (*Thuja occidentalis* 'Smaragd') with partly dead foliage at our plant clinic. It was approximately a meter high and was one of 45 plants that had been established in a hedgerow in Oslo in April 2011. By July 2011, five plants had to be replaced due to severe disease symptoms. The plants had been imported via a garden center. There was no canker symptom in the stem base of the plant we received, but the roots had typical dieback symptoms; fine roots were absent and the roots were clearly discolored below the bark. A *Phytophthora* sp. was isolated from the roots. It was identified as *P. pini* by ITS sequencing. This species was first described in 1925, isolated from roots of *Pinus resinosa* in Minnesota, USA. Later the species was merged with *P. citricola*, but has recently been segregated again from the *P. citricola* complex [1]. It has mostly been reported from USA, but also from nurseries in Europe. Among other hosts it has been reported from *Fagus sylvatica*, *Rhododendron* sp., and *Thuja* sp. It has also been isolated from streams and irrigation water [1, 2]. A pathogenicity test was started on 27 March 2012.

[1] Hong, C., Gallegly, M. E., Richardson, P. A. and Kong, P. *Phytophthora pini* Leonian resurrected to distinct species status. *Mycologia*, **103** 351-360 (2011).

[2] Jung, T. and Burgess T. I. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia*, **22** 95–110 (2009).

Disease management of *Phytophthora ramorum* in a research quarantine nursery at NORS-DUC

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Sudden Oak Death, caused by *Phytophthora ramorum* Werres, De Cock & Man in't Veld, is among the most important emerging forest diseases with potentially devastating effects on temperate forests. The nursery trade is a factor in the long-range spread of *P. ramorum*. To address threats posed by plant trade and aid in developing an environmentally-sustainable nursery industry, the National Ornamentals Research Site at Dominican University of California (NORS-DUC) was developed (www.dominican.edu/norsduc). NORS-DUC is a sophisticated field nursery designed to contain quarantined pathogens for the purpose of conducting research in a safe environment that reflects an authentic nursery setting. Research currently focuses on *P. ramorum* and is conducted by a consortium of *P. ramorum* experts. NORS-DUC offers an unparalleled opportunity to study the epidemiology of *P. ramorum* diseases of ornamentals that has not been accomplished previously. First results from research on the disinfection of *P. ramorum* from field soils using steam sterilization and biocontrol using a new *Trichoderma* isolate were effective and these methods are being reviewed by USDA APHIS for soil



treatment in infected commercial nurseries. Additional research is advancing our understanding of the affect of fungicides on symptom development and *P. ramorum* lifestages. Upcoming research will contribute to our understanding of disease epidemiology in nurseries to provide meaningful data to aid design of effective pest management programs. NORS-DUC is expected to continue contributing towards solutions for containment, remediation, and eradication of quarantine pathogens in nurseries, reducing the risk of long-range spread of pests through infested nursery stock shipments.

Poster 30

Heat-treated Japanese larch (*Larix keampferi*) wood chips can counter persistence of *Phytophthora ramorum*

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Phytophthora ramorum is the causal agent of 'sudden oak death' in North America. In the British Isles it infects primarily rhododendron but since 2009 has spread to Japanese larch infecting and killing thousands of trees [1]. Novel methods are being sought to combat the disease including the potential of wood chip mulches to reduce the survival and spread of the pathogen. Previous studies have shown that heating wood shavings obtained from a variety of tree species causes the shavings to become highly anti-microbial, with activity against both bacteria and fungi [2]. The aim of this study was to investigate the antimicrobial activity of heat treated wood materials against *P. ramorum* focussing on the most frequently infected hosts, rhododendron and larch.

Samples of Japanese larch and rhododendron wood were chipped and heat-treated at 140° C for three days. GC-MS analysis of methanol crude extract of pre- and post-heated chips identified anti-microbial chemicals, particularly in the larch, that could have activity against *P. ramorum*. To test this, colonised leaf disks of rhododendron were treated with the methanol crude extract derived from the heated woodchips and it prevented any growth of *P. ramorum*, including chlamydospore germination. Subsequently, treated and untreated wood chips were tested in a micro-cosm system, by challenging with zoospores of *P. ramorum*. It was found that the effectiveness of the anti-microbial action of the wood chips altered with wood type and pathogen genotype. However, the approach shows promise for reducing spread/persistence of *P. ramorum* on affected sites with the treated chips used as a pathogen-suppressive mulch in gardens and areas with limited infection.

[1] Webber, J. F., Mullett, M., Brasier, C. M. Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum*. *New Disease Reports* **22** 19 (2010). [<http://dx.doi.org/10.5197/j.2044-0588.2010.022.019>]

[2] De Leij, Franciscus and Mina Kalantarzadeh. "Antimicrobial material." US patent 0034289 A1. 09 Feb 2009.

Poster 31

Development of molecular markers and probes for detection of *P. ramorum*, *P. nicotianae*, *P. citricola*, *P. fragariae* and *P. cactorum*

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Phytophthora diseases are some of the most dangerous diseases of trees and shrubs including raspberry and strawberry. Root rot of raspberry and strawberry, caused by *P. fragariae*, and sudden oak death,



caused by *P. ramorum* should be given particular attention. These pathogens are regulated as quarantine pests in many countries, including Russia.

Laboratory diagnosis of these oomycetes is complicated due to similarity of symptoms caused by the pathogens as well as due to variability of their morphological characteristics. The importance and practical relevance of this study lies in the necessity for developing and further improving molecular methods for diagnosing *Phytophthora* diseases.

In developing real-time PCR, we selected primers and probes using Ypt gene [1]. Nucleotide sequences of *Phytophthora* spp. Ypt gene were picked out from the GenBank and sequencing of pure cultures from the All-Russian Plant Quarantine Center Reference Collection.

For diagnosing sudden oak death, we selected *P. ramorum* - specific PramF and PramR primers, and PramP probe marked with MGB fluorescent dye.

In developing multiplex real-time PCR for differentiating among major causal agents of raspberry and strawberry root rot, we selected universal primers for *Phytophthora* spp., and probes marked with TAMRA fluorescent dye specific for *P. cactorum*, *P. fragariae*, *P. nicotianae*, *P. citricola*.

Primers and probes marked with various fluorescent dyes allowed real-time PCR for detection and identification of the four oomycetes species in a single tube. Selected primers (PramF, PramR) and the probe PramP did not cross react with other closely-related *Phytophthora* spp. causing tree diseases.

[1] Schena L., Duncan, J. M. and Cooke, D. E. L. Development and application of a PCR-based 'molecular tool box' for the identification of *Phytophthora* species damaging forests and natural ecosystems. *Plant Pathology*, **57** 64-75 (2007).

Poster 32

Seasonal variation of inoculum density and species composition of soilborne *Phytophthoras* in an infected black walnut stand in Hungary. J. Kovács, F. Lakatos, I. Szabó

[See Session 3](#)

Poster 33

Fluorescent in situ hybridization (FISH) assay as a tool to microscopically view *Phytophthora cinnamomi* growth within plant tissues. A. Li, M. Crone, P. Adams, S. Fenwick, N. Williams, G. Hardy

[See Session 6](#)

Poster 34

Early *Phytophthora* spp. detection by qPCR

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One of the main European nursery poles for ornamental plants is located in Pistoia, Tuscany, Italy, where nurseries are spread over more than 5300 hectares. The production is exclusively devoted to the production of plants in pots. In average, 80% of plant obtained by Pistoia's nurseries is exported to foreign countries. For this reason the production of healthy material is fundamental, in order to avoid the spread of nasty pathogens all over the world.



Among the diseases affecting plants in nursery, the root rots are one of the most important, particularly those caused by *Phytophthora* species. Since this class of pathogens is hard to isolate, and remains alive in the soil for long time periods before causing symptoms, the availability of an early detection tool is of primary concern to prevent the risk of spread of pathogens.

Aim of this work was to develop a real time PCR assay to detect and quantify *Phytophthora* spp. from plant and soil samples collected in nurseries in Tuscany. The survey was carried out by collecting plants in pots and soil samples. Isolation on selective media according to Moralejo et al [1] and DNA extraction were performed on leaves, roots and soil of both symptomatic and asymptomatic samples.

The sensitivity and specificity of real time PCR approach make possible to detect the presence of small amount of *Phytophthora* DNA in soil and plant samples even before symptoms occur.

The use of this molecular tool will prevent the spread of hitch-hiker pathogens by asymptomatic plants in anthropic and natural ecosystems.

[1] Moralejo, E., Pérez-Sierra, A., Álvarez, L. A. Belbahri, L., Lefort, F. and Descals, E. Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain. *Plant Pathology*, **58** 100-110 (2009).

Poster 35

Diversity of *Phytophthora* species in forest ecosystems of Bulgaria

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The fungus-like organisms from the genus *Phytophthora* currently include more than 100 species, many of which are destructive plant pathogens. The most aggressive *Phytophthora* species are now acknowledged as causal agents in several devastating declines of forest trees in Europe, Australia and the USA. Therefore, *Phytophthora* surveys are carried out by scientists and forest services in most of the European countries. Despite the fact that Bulgaria is rich of natural ecosystems comprehensive information about the occurrence and diversity of *Phytophthora* species in forests and forest nurseries is still missing. The purpose of this study is to fill in this gap by detecting and identifying *Phytophthora* pathogens potentially dangerous for the Bulgarian forests ecosystems. Samples were primarily collected from regions that are close to the country borders and areas along major transportation roads, as *Phytophthora* pathogens are known to be distributed via trading, transportation and other human activities. The focus of our study was on known hosts of *Phytophthora* such as oak, sweet chestnut, alder, spruce etc. growing in forest ecosystems and nurseries. More than 100 samples of rhizosphere soil and plant tissue from trees with typical disease symptoms have been collected and examined for the presence of *Phytophthora*. Several *Phytophthora* isolates were obtained and their species identity was determined on the basis of their morphological, cultural and molecular characteristics. Up to now *P. cryptogea*, *P. cambivora*, *P. plurivora* and *P. rosacearum* were detected.



Determination of the minimum threshold of *Phytophthora cinnamomi* inoculum for infection of *Quercus* spp.

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The root rot due to *Phytophthora cinnamomi* is the main cause of the massive death of Holm and cork oaks affecting rangeland ecosystems in the south of the Iberian Peninsula. The knowledge of the minimum level of inoculum of the pathogen in the soil for infection of tree roots is a crucial point for risk assessment. To determine this minimum level of inoculum for infection of *Quercus ilex* spp. *ballota* and *Q. suber* roots, 18 months-old seedlings were planted in a substrate previously infested with aqueous suspensions of chlamydospores of two different isolates of *P. cinnamomi* (PE90 and PA25 isolated from roots of Holm and cork oaks, respectively) in increasing concentrations (0, 0.6, 6, 60, 600 and 6000 chlamydospores per gram of dry soil). Ten plants (repetitions) were planted per inoculum concentration and species of *Quercus*. All the plants were incubated in air-conditioned greenhouse at 25-10° C day/night, maintaining soil flooding for 2 days per week. Weekly, severity of foliar symptoms is evaluated on a 0-4 scale (0 = 0-10% of symptomatic tissue, 4 = dead tissue) and at the end of the experiment, the severity of radical symptoms will be assessed following the same scale. The results obtained for both species of *Quercus* will be discussed at the meeting.

Could climate change influence the survival of *Quercus ilex* seedlings germinating in *Phytophthora* infested soils?

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Holm oak “dehesas” are amongst the most representative forest ecosystems in the Iberian Peninsula. Over the last three decades a serious forest decline associated with several species of *Phytophthora* has been observed. Although previous reports describe the pathogenicity of different species of *Phytophthora* to *Quercus* spp. in this ecosystem [1], empirical evidence concerning the possible responses of *Phytophthora* species to climate change are lacking. An increase in temperature between 2 - 4.5° C (with a most likely value of 3° C) is expected in the 21st century [2], which will lead to changing interactions between vegetation and pathogens and to a variety of forest health problems. The aim of this work was to determine whether an increase in temperatures would influence the germination and survival of *Quercus ilex* seedlings growing in *Phytophthora* infested soils. Acorns of *Quercus ilex* (provenance: Malpartida de Plasencia, Spain) were stored at 4° C before sowing. Inocula of *P. cinnamomi*, *P. gonapodyides*, *P. quercina* and *P. psychrophila* were raised on vermiculite - oat - V8 juice medium at 20° C for 6 weeks. Four hundred acorns were sown in infested soils with each of the four *Phytophthora* species separately, plus a control treatment of non-infested soil. Two temperature treatments were used: 17 or 20° C with the same photoperiod (n = 40 replicates/treatment). Germination, mortality rates, plant growth (above-ground part and root system) and photosynthetic capacity data will be presented.

[1] Sánchez, M.E., Andicoberry, S. and Trapero, A. Pathogenicity of three *Phytophthora* spp. causing late seedling rot of *Quercus ilex* ssp. *ballota*. *For. Path.*, **35** 115–125 (2005).



[2] IPCC. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). [Cambridge University Press](#), Cambridge, United Kingdom and New York, NY, USA (2007).

Poster 38

A new method to quantify zoospore chemo-attraction. N.S. Massola JR., R. Dalio, F. Fleischmann, W. Osswald

See Session 6

Poster 39

Cryptogein and capsaicin elicit defence responses in cork oak

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Cork oak (*Quercus suber*) decline in Iberian Peninsula stands is associated with infection by *Phytophthora cinnamomi*. Most *Phytophthora* species secrete elicitors, which can enhance defence reactions against some pathogens.

This work recently published on-line [1], is the first to report a potentially protective role of *Phytophthora*- derived elicitors against *P.* infection in a *Fagaceae* (*Q. suber*). It highlights the effect of cryptogein and capsaicin (secreted by *P. cryptogae* and *P. capsici*, respectively) on the infection of *Q. suber* roots by *P. cinnamomi*.

Cytological and physiological effects of the two elicitors on cork oak root infection by *P. cinnamomi* were evaluated. The progression of the pathogen in root tissue and its effects on total fatty acid (TFA) of roots and leaves were analysed in seedlings. Net photosynthesis (Pn), stomatal conductance (gs), chlorophyll a fluorescence (quantum yield of linear electron transport ϕ , photochemical quenching qP, non-photochemical quenching NPQ) and carotenoid determinations were carried out in 4-month-old plants. In elicitor-treated roots, 2 days after inoculation, the pathogen was mainly restricted to the intercellular spaces of the cortical parenchyma, and did not reach the vascular cylinder of roots. Electron dense materials accumulated in the intercellular spaces of the cortex next to disorganized hyphae, suggesting to be related with defence reactions. Cryptogein (or its interaction with *P. cinnamomi*) induced enhanced lipid synthesis in leaves. *P.cinnamomi* decreased Pn, gs, ϕ , and qP, whereas elicitor treated plants displayed values similar to controls. Results indicated a resistance response of cork oak against this oomycete, induced by the elicitors.

[1] Medeira, C., Quartin, V., Maia, I., Diniz, I., Matos, M.C., Semedo, J., Campos, P., Ramalho, J., Pais, I., Ramos, P., Melo, E., Leitão, A., Cravador, A. Cryptogein and capsaicin promote defence responses and photosynthesis protection in *Quercus suber* against *Phytophthora cinnamomi* infection. *European Journal of Plant Pathology* DOI 10.1007/s10658-012-9972 (2012).



***Phytophthora* species in Serbia.** I. Milenkovic, N. Keca, J. Nowakowska, K. Sikora, M. Borys, T. Oszako, T. Jung

See Session 5

Susceptibility of *Quercus ilex* to mixed infections by multiple *Phytophthora* species

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Oak decline in Iberia is a complex phenomenon that requires the combination of several factors to occur. Although declining trees are often damaged by several pathogenic oomycetes [1, 2] the interaction among these organisms has still not been investigated. The present study aims to determine under greenhouse conditions whether possible interactions among different species of *Phytophthora* might have significant synergistic effects on *Quercus ilex* decline. The material comprised 1-year-old *Q. ilex* seedlings grown from acorns of a single tree (Malpartida de Plasencia, Spain), and single strains of *P. cinnamomi* (C), *P. gonapodyides* (G) and *P. quercina* (Q) isolated from rhizosphere soil of declining *Q. ilex* trees. Inocula were prepared by growing the *Phytophthora* strains in vermiculite - oat seeds - V8 juice medium at 20°C for 6 weeks. Repeated inoculations were performed in December 2011 and in January 2012, so that nine combinations (CC, GG, QQ, CG, CQ, GC, GQ, QC, QG) plus a control treatment of non-inoculated seedlings were tested (n=22 seedlings). All plants were submitted to a regime of two days waterlogging per week. Mortality rates in April 2012 were 100, 36, 9, 95, 95, 64, 50, 86, 9, and 0% for CC, GG, QQ, CG, CQ, GC, GQ, QC, QG and control treatments, respectively. Inoculation of C, G or Q did not enhance plant resistance to a second inoculation, and no synergism or antagonism effects among *Phytophthora* species were observed. As a preliminary conclusion, highest mortality of seedlings does occur when *P. cinnamomi* is present, irrespectively of the inoculation date.

[1] Corcobado, T., Cubera, E., Pérez-Sierra, A., Jung, T. and Solla, A. First report of *Phytophthora gonapodyides* involved in the decline of *Quercus ilex* in xeric conditions in Spain. *New Disease Reports*, **22** 33-33 (2010).

[2] Pérez-Sierra, A., López-García, C., León, M., García-Jiménez, J., Abad-Campos, P. and Jung T. Detection of *Phytophthora* spp. on *Quercus* spp. in the Natural Park Carrascar de la Font Roja (preliminary). Proceedings of the COST Action FP0801 "Established and Emerging *Phytophthora*: Increasing threats to Woodland and Forest Ecosystems in Europe" (2011).



Effect of *Phytophthora quercina*, *P. gonapodyides* and *P. cinnamomi* on germination of *Quercus ilex* acorns and seedling establishment in infested soils

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In Iberia, several species of *Phytophthora* are involved in the decline of *Quercus ilex*. The pathogen *P. cinnamomi* is the most frequent species isolated, whereas *P. quercina* and *P. gonapodyides* are less detected [1]. Natural regeneration of declining holm oaks is a main goal for the forest administration, but no information is available about the viability of germinating acorns and subsequent establishment of seedlings in infested soils. Previous research reported 100% mortality of *Q. ilex* seedlings when pre-germinated seeds were placed into soils infested with *P. cinnamomi* [2]. Our objectives were to quantify the establishment of *Q. ilex* through acorns directly sown into infested soils, and to detect possible differences between different tree provenances. In December 2011, acorns were collected and stored at 4°C for two months, and then were sown 1 cm deep into a mixture of sand and peat (1:1) containing inoculum of the three pathogens, separately. Non-infested soil mixture was used as control. The presence of *P. cinnamomi* significantly reduced *Q. ilex* germination, and afterwards caused about 50% of seedling mortality (60 dpi). The presence of *P. quercina* and *P. gonapodyides* did not alter germination but significantly reduced fine root growth and the tap root length in comparison to plants growing in non-infested soil. Several traits assessed among the plants from different provenances revealed interesting *Phytophthora* spp. x plant origin interactions. It is concluded that the presence of *Phytophthora* spp. is clearly a limiting factor in natural regeneration processes.

[1] Corcobado, T., Cubera, E., Pérez-Sierra, A., Jung T. and Solla A. First report of *Phytophthora gonapodyides* involved in the decline of *Quercus ilex* in xeric conditions in Spain. *New Disease Reports*, **22** 33-33 (2010).

[2] Rodríguez-Molina, M. C., Torres-Vila, L. M., Blanco-Santos, A., Palo-Núñez, E. J. and Torres-Álvarez, E. Viability of holm and cork oak seedlings from acorns sown in soils naturally infected with *Phytophthora cinnamomi*. *Forest Pathology*, **32** 365–372 (2002).

In vitro inhibition of mycelial growth of *Phytophthora cinnamomi* by pellets of brassicas

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Phytophthora cinnamomi is considered one of the main agent involved in oak decline, a phytosanitary problem currently affecting Spanish forests and *dehesas* (Mediterranean open woodlands). Biofumigation is an environmentally friendly method of controlling soil pathogens, so it may be a valid alternative to chemical use in agroforestry and *dehesas* and also economically viable. The hydrolysis products of glucosinolates in the brassicas tissues are potentially useful to control fungal pathogens. In this *in vitro* study there was evaluated the effect of the pellets of brassicas (BioFence®) on the inhibition of mycelial growth of *P. cinnamomi*. Eight biofumigant concentrations were tested (5, 7.5, 10, 12.5, 15, 20, 40 and 60 mg; 40% humidity) on ten isolates of *P. cinnamomi* to calculate by Probit analysis the EC₅₀



and EC₉₀. Plugs of PDA with actively growing mycelium were cut and transferred to the centre of petri plates containing PARP-V8 juice agar. All plates were incubated at 25°C for 24h before being exposed to the biofumigant material. Biofumigant material was placed on the cover of petri plate and the plates were incubated inverted in the dark at 25°C for 72h. After that time, radial growth was measured taking two perpendicular diameter measurements for each colony. All isolates assayed showed to be highly susceptible to the effect of biofumigant although differed in their susceptibility, with EC₅₀ values ranging 5.59 and 14.65 mg/plate and EC₉₀ values ranging 14.97 and 28.10 mg/plate. This biofumigant is presented as an option for *P. cinnamomi* control although bioassays under controlled conditions are necessary before using at oak decline sites.

Poster 44

PCR detection of *Phytophthora ramorum* from woody tissue

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Rapid and accurate detection of *Phytophthora ramorum* from advisory and quarantine samples is a key step in managing the spread of this pathogen. Along with standard culturing methods, PCR and real time PCR are effective tools for confirming infection, but in the case of woody hosts such as larch (*Larix* sp) these methods can have a low success rate, giving rise to false negatives and delays in managing disease outbreaks.

Comparison of different target genes were made including the use of the Elicitin gene and the ITS region. Significant differences were found in the CT values gained from the two assays compared.

A range of alternative extraction protocols and commercial kits were tested with varying degrees of PCR inhibitor removal. One kit consistently out performed the others. Further optimisation of this kit was carried out by modification of protocols and proportions of reagents. Additional components were also tested for their effectiveness in conjunction with the preferred extraction kit. Significant improvements were achieved by the adoption of these changes, resulting in a protocol with improved detection rates through increased efficiency of extraction from difficult tissues.

Comprehensive testing of available PCR reagents also resulted in improved detection of target. An increase in sensitivity was achieved and a significant reduction in false negatives being reported.

Poster 45

Phytophthora plurivora and *Phytophthora multivora* in the Czech Republic – comparison of distribution and pathogenicity to forest trees

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The two new pathogens – *Phytophthora plurivora* and *P. multivora* – were described in *P. citricola* complex [1, 2]. *P. plurivora* is widespread in forest, semi-natural ecosystems and nurseries across Europe [1], whereas *P. multivora* is responsible for damages in natural ecosystems in Western Australia [2], but it was found in Europe [1] and also in the Czech Republic.



It was found out, that *P. plurivora* predominated over *P. multivora* (4/5 of isolates came under *P. plurivora*) in the investigated area and it was distributed in broad spectrum of altitude (162 – 611 m a.s.l.). The other species was rarely found and only in the lowest (up to 216 m a.s.l.) and most warm locations. Both species were found in nurseries, parks, forest and riparian stands and were isolated from oak, alder, willow and rhododendron. Moreover, *P. plurivora* was acquired from 13 other hosts. The morphological analysis revealed that Czech isolates of *P. multivora* differed from the Australian ones by thinner oospore wall and lower OWI. Likely, it can be an adaptation to the more friendly climate in Central Europe in comparison with Western Australia.

The pathogenicity test with 6 native forest trees (beech, oak, etc.) revealed that *P. plurivora* is more aggressive than *P. multivora*, but *P. multivora* also posed an important risk to European forests. Substrate specificity was detected in *P. plurivora* – the isolates from forests trees were more aggressive to them than the isolates from ericaceous plants. Likely, the both subpopulations (in anthropogenous and natural stands) were partially isolated and specialised to the accessible hosts.

[1] Jung, T. and Burgess, T.I. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* **22** 95–110 (2009).

[2] Scott, P.M., Burgess, T.I., Barber, P.A., Shearer, B.L., Stukely, M.J.C., Hardy, G.E.StJ. and Jung, T. *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia* **22** 1–13 (2009).

Poster 46

***Oomycete* survey at two cork oak stands at Alentejo. Correlation with the declining condition**

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The aim of this work is to make a survey for the presence of phytopathogens in cork oak roots and associated soil in typical productive Montado Ecosystems. It is part of a wider integrative study aiming to assess the risk and the ecological sustainability of these ecosystems (RESCOE - PTDC/BIA-BEC/102834/2008). Two typical montados in Alto Alentejo, Herdade do Freixo do Meio (FM) and Gouveia de Baixo (GB) were selected and in each one two sub-areas defined according to contrasting tree aerial aspect: sub-area A with healthy, and sub-area B with declining cork oak trees and/or associated tree mortality. In each sub-area 10 healthy and 10 declining trees, were selected. The trees were labelled, photographed and GPS located in order to allow their easy identification throughout the project. Their symptoms were evaluated and the trees ranked according to their degree of defoliation: Class 0 = no symptoms (1-10% of defoliation); Class 1 = 11-25%; Class 2 = 26-60 %; Class 3 = 61-90%; Class 4 = 91-100% and trees that have died suddenly with or without leaf loss. This work has been carried out since October 2010, and samples are collected every autumn and Spring. Soil samples from the rhizosphere of each selected tree were collected. The isolation of the oomycetes was achieved by using biological baits, such as young leaves of *Q. suber* and *Q. ilex*. Briefly, 1000 ml of soil from each sample were baited with cork oak leaves. Necrosed leaves were cut, plated in selective medium (NARPH). Then each colony was observed in a light microscope and the ones that were morphologically distinct were transferred to NARPH or directly to V8 agar (V8A) to be identified. Several oomycetes were identified by ITS sequencing. *P. cinnamomi* was isolated from 73.3% and 6,7%, GBB and FMB samples respectively. Six *Pythium* species were also isolated, of which only *Pythium spiculum* is known to be pathogenic for cork oak, to a less extent then *P. cinnamomi*. *Py. spiculum* was isolated from GBA and GBB sub-areas. These results show that *P. cinnamomi* is the only aggressive soil born pathogen present in declining areas. The healthy state of the trees appears to correlate with its presence. The average



degree of defoliation has increased in all sub-areas over time. *P. cinnamomi*, is an exotic highly aggressive pathogen that has not co-evolved with cork oaks, and therefore the trees have limited defenses against it. Thus, we would expect trees from sub-areas B to continue to decline.

Poster 47

Looking for a new quick and effective method of *Phytophthora* identification in environmental samples

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Contemporary forest protection needs a rapid and reliable method of identification of new invasive species both in nurseries and forest stands. Analysis of soil and water samples supplied a significant information about occurrence of fine root pathogens in Poland. Traditional baiting and morphological observations complement with physiological test and DNA analysis. Designing of real time probes specific to pathogen has advantages in nursery trials or pathological tests worth considering. So far specific probes designed for *P. plurivora*, *P. cactorum*, *P. alni*, *P. cambivora*, *P. quercina* and *P. pseudosyringae* were successfully tested. Two more probes prepared for *P. citrophthora* and *P. cryptogea* need to be improved as they are not specific and cross with other *Phytophthora* species. Some new techniques like Padlock probe amplification followed by micro-array analysis, Fluidigm amplification followed by 454 sequencing and Luminex multiplex analysis are tested or are under study.

Poster 48

The occurrence of *Phytophthora* species in European Ecological Network NATURA 2000 in Poland

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The research was done in Western (along Oder river) and West-Central (Krotoszyn Plateau) parts of Poland. DNA was extracted directly from soil samples (pre-incubation method) and from water (which was first filtered). In oak stands *Phytophthora quercina* and *P. plurivora* were the most common species causing damaged to fine roots. *P. cactorum* and *P. pseudosyringae* were detected, too. This put the new light on oak decline phenomenon which has been occurring in these regions since the 80's.

Poster 49

Roads and streams are not significant pathways for SOD spread in tanoak forests. E. Peterson, J. Hulbert, E. Hansen

See Session 3



Ecophysiological reactions of alder (*Alnus* spp.) after the infection with *Phytophthora alni* ssp. *alni* in the field and during controlled green-house conditions

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The new hybrid *Phytophthora alni* sp. nov. is spreading in Germany. Along rivers and creeks, surrounding bogs and lakes *Alnus glutinosa* is showing the classic symptoms of leaf desiccation and crown defoliation as well as stem cankers and bleeding. Many of the infected trees die within the following years. To study the ecophysiological effects of *P. alni* on *A. glutinosa* experiments with intact twigs, intact detached leaves and seedlings were performed in the field as well as in the laboratory. It was shown that infected trees have less and smaller leaves with a clearly reduced chlorophyll content. Photosynthetic capacity, measured either as electron transport capacity (ETR) via Chl-fluorescence or by CO₂ gas exchange was clearly reduced in infected trees. Also cortical photosynthesis was affected around artificial inoculations of stems. Following anatomical changes around the inoculation, also the chlorophyll content of the inner bark was drastically reduced. ETR measurements underlined this fact. Field observations showed a dramatic spreading of the stem infection within months. Absorptivity measurements showed clear changes in light absorption of infected and uninfected tissues of the stems. When seedlings or detached twigs were fed with extracts from infected trees, leaf wilting was observed.

***Phytophthora ramorum* and *P. lateralis* in Northern Ireland.** L. Quinn, A. Mccracken, B. Moreland

See Session 5

Histological changes of *Quercus ilex* seedlings infected by *Phytophthora cinnamomi*. M.A. Redondo, A. Pérez-Sierra, P. Abad-Campos, J. García-Jiménez, A. Solla, J. Reig, F. García-Breijo

See Session 6

A new *Phytophthora* species in ITS Clade 2 killing *Ceanothus* grown for rehabilitation of disturbed forest sites

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Three species of *Phytophthora*, comprising *P. cactorum*, *P. pini*, and an undescribed species related to *P. himalsilva* and *P. citrophthora*, were isolated from stems and roots of dying *Ceanothus* plants (*C. sanguineus*, *C. integerrimus* and *C. velutinus*) being grown at a native plant nursery for transplanting to a



mine rehabilitation site in the Siskiyou Mountains of Oregon, USA. *Ceanothus* is a genus comprising around 60 species of small trees and shrubs which are important components of many wildland ecosystems in North America. Of 20 symptomatic *Ceanothus* plants sampled, one plant yielded *P. pini*, 2 plants yielded *P. cactorum*, and 12 plants yielded the undescribed species. Pathogenicity testing is underway, but it was evident from a subsequent nursery visit that damage was severe and cultural practices favored *Phytophthora* infection. To prevent similar occurrences in the future, native plant nurseries (making unregulated in-State sales) must conform to the same Best Management Practices currently used by many larger nurseries (certified for interstate shipping) to assure production of disease free planting stock. Botanists charged with forest restoration must demand healthy stock from nurseries in the same manner as regeneration foresters now demand quality from forest tree nurseries.

Poster 54

***Phytophthora* ITS Clade 3 expands to include a sixth new species, *P. taxon pluvialis*.** P. W. Reeser, W. Sutton, E.M. Hansen

See Session 5

Poster 55

Evaluation of biofumigant plants for control of *Quercus* root rot caused by *Phytophthora cinnamomi* in rangeland ecosystems

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Root rot caused by *Phytophthora cinnamomi* is the most serious disease affecting *Quercus ilex* and *Q. suber* in oak rangeland ecosystems in southern Spain. A suitable strategy for disease control in these seminatural ecosystems is the use of biofumigant crops able to inhibit *P. cinnamomi* infections. Effects of two different genotypes of three potential biofumigant species (*Brassica carinata*, *B. juncea*, *B. napus*) on pathogen mycelial growth have been studied. Fresh plant material was collected at different phenological stages (stem extension and flowering) and macerate for direct testing, or collected and lyophilized before testing. Cultures of the pathogen plated on Carrot-Agar media were exposed to plant material (fresh or lyophilized and rehydrated) at different doses: 0 g (control), 5 g, 10 g and 20 g per plate and incubated in growth chamber at 25° C in the dark. Four different replicates were prepared for biofumigant plant material and dose. Two colony radiuses were daily measured. Data obtained were analyzed (ANOVA) and average values compared among them and with controls by Tukey's test. At the same time, glucosinolate content is being analyzed for each species, genotype and phenology tested by EU reference method (HPLC of desulphoglucosinolates).

All biofumigant treatments reduced mycelial growth of *P. cinnamomi*, but complete suppression was reached by all doses of both genotypes of *B. carinata* and *B. juncea*. A higher number of potentially biofumigant plants should be tested in order to choose the most effective ones for testing their ability to decrease the inoculum potential for root infections in artificially and naturally infested soils.



Variation in pathogenicity of *Phytophthora lateralis* and interactions with its host *Chamaecyparis lawsoniana*.

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Phytophthora lateralis is an invasive pathogen which has had a severe impact in Oregon and California and which is nowadays reported in Europe (first in France and the Netherlands [1], then in Scotland). Additionally, in 2008, *P. lateralis* was detected in soil of natural old growth forests of *C. obtusa* in Taiwan, in agreement with a possible Asiatic origin for this species.

The principal host, *Chamaecyparis lawsoniana*, of *P. lateralis* is very common in Europe and grown in nurseries, where the disease can spread very easily as observed in Oregon in the 50s. *P. lateralis* is thus considered as a high risk species which should be considered with caution and for which we need to develop management methods.

Several cedar trees that have survived natural epidemics or in artificially infested raised beds have been tested for their resistance to *P. lateralis* using different inoculation procedures [2]. A program was initiated by USDA Forest Service in cooperation with Oregon State University to derive benefits of this genetic resistance which appeared to occur in *C. lawsoniana* and to use resistant trees in seed orchards.

We carried out artificial inoculations of *C. lawsoniana* trees with French, Taiwan and American isolates. Differences in aggressiveness between *P. lateralis* isolates were expressed with the stem-wound inoculation technique and the root dip test. Specific genotype x genotype interactions will be analysed with an inoculation test of five progenies of resistant or susceptible parents. This study should offer interesting insights into the durability of resistance in *C. lawsoniana* trees.

[1] Robin, C., Piou D., Feau N., Douzon G., Schenk N. and Hansen E.M. Root and aerial infections of *Chamaecyparis lawsoniana* by *Phytophthora lateralis* : a new threat for European countries. *Forest Pathology*, **41** 417–424 (2011).

[2] Hansen, E.M., Hamm, P.B. and Roth, L.F. Testing Port-Orford-Cedar for resistance to *Phytophthora*. *Plant Disease*, **73** 791-794 (1989).

Evaluation of fungal secondary metabolites against *Phytophthora* spp.

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Phytophthora is the major genus within the Oomycota and it is considered one of the most serious threats for forest and natural ecosystems on a global scale. Within the disease management strategies, there is a need for new effective compounds owing to the development of fungicide resistance by pathogens and adverse effects on environmental ecosystems. Bioactive secondary metabolites of microbial origin, which have historically been of great importance in medicine and agriculture, could be expected to overcome these critical points. In this study, seven bioactive metabolites produced by phytopathogenic fungi and/or biocontrol agents were examined against several pathogenic species of *Phytophthora* isolated in natural ecosystems in Sardinia (Italy). The effects of these metabolites were assessed *in vitro* on mycelial growth, sporulation, oospores formation and zoospores motility. The fungicide metalaxyl-M (48% active ingredient) was used as positive control. All metabolites were



assayed at increasing concentrations and EC₅₀ values were evaluated. Interestingly, mycelial growth inhibition was observed with sphaeropsidin A, which showed to be the most active metabolites. The EC₅₀ for sphaeropsidin A was ranging 1-10 µg/mL depending on the *Phytophthora* species, and it was tenfold higher than that of metalaxyl-M in some species. The EC₅₀ for the other metabolites tested exceeded 100 µg/mL. Although the chemical control of these pathogens is impractical in natural ecosystems, our results may provide a basis for the development of new compounds that may have useful applications in agriculture and nurseries.

Poster 58

Evidence of lipophilic phytotoxic metabolites produced by *Phytophthora* spp. involved in chestnut ink disease

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Several species of *Phytophthora* have been proven to be involved in the chestnut ink disease. The nature of symptoms caused by these pathogens suggests that phytotoxic metabolites might be involved in the host-pathogen interaction. Furthermore, *Phytophthora* species are known to secrete proteins which play a key role in the host-pathogen interaction. Three selected isolates of *P. cambivora*, *P. gonapodyides* and *P. pseudosyringae* were examined for their ability to produce *in vitro* phytotoxic secondary metabolites. Cultures were grown in Roux bottles containing a defined liquid medium, and incubated in steady conditions at 21°C at the dark. After 30 days cultures were filtered using MF-Millipore™ membrane filters. In order to obtain the active lipophilic compounds, culture filtrates were extracted exhaustively with ethyl acetate. Culture filtrates and organic extracts along with the exhausted aqueous phase were tested on 20 days-old tomato cuttings. Culture filtrates induced symptoms of wilting after 3 days from the treatment up to 10% dilution. All organic extracts resulted active in the bioassay trials, suggesting that lipophilic metabolites could be involved in host-pathogens interaction in addition to hydrophilic metabolites. The isolation and purification of virulence factors of these *Phytophthora* species are in progress. To our knowledge, this is the first report regarding the production of lipophilic compounds by *Phytophthora* species.

Poster 59

Phytophthora species occurring in declining oak ecosystems in Sardinia (Italy)

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Quercus ilex and *Quercus suber* are the main forest species in Sardinia. Since 2009 a survey on the occurrence of *Phytophthora* species has been conducted in ten oak stands. Rhizosphere soil samples were collected from symptomatic oak trees and baited using oak leaflets. In addition, bark samples were taken from lesions and cankers present on stems. Isolations were made using SMA selective medium for *Phytophthora*. Isolates were identified based on morphological characters, growth rates, cardinal temperatures for growth and ITS sequence analysis. Six *Phytophthora* species were identified, including *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. gonapodyides*, *P. psychrophila* and *P. quercina*. Two unusual *Phytophthora* species are still in phase of identification since their morphological and molecular properties did not match any formally described species or informally designated taxon. *Phytophthora cinnamomi* was the most frequently isolated species. It was particularly found associated with severe decline of *Q. suber* trees. The oak-specific *P. quercina* was detected only at one site where it was causing



extensive dieback of *Q. ilex* trees. The isolations of *P. citrophthora*, *P. cryptogea* and *P. psychrophila* from rhizosphere soil represent the first records of these species in *Q. ilex* and *Q. suber* stands in Italy. Pathogenicity tests are in progress in order to assess the susceptibility of both oak species to all eight *Phytophthora* species/taxa. The occurrence of some unidentified *Phytophthora* spp. suggests that many aspects related to diversity of *Phytophthora* in Sardinia and their role in the decline of oaks remain unexplored and further research is urgently required.

Poster 60

Development, comparison and validation of a Real-Time PCR tool for the detection of *Phytophthora lateralis*

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Outbreaks of *Phytophthora lateralis* were recently identified in north-western France (Brittany) on Port-Orford Cedar trees (*Chamaecyparis lawsoniana*) planted as hedgerows in the 1970s [1]. This soil and airborne aggressive oomycete represents a new and serious threat to European countries.

Therefore, rapid, specific and sensitive detection of the pathogen is essential. A new *in planta* detection protocol based on real-time polymerase chain reaction was developed. A *P. lateralis*-specific combination of primers and hydrolysis probe has been designed in the RAS-Ypt gene, in regions showing interspecific polymorphisms [2]. This new test proved to be sensitive and highly specific since it does not cross react with the closely related species *P. ramorum*. The relative accuracy, specificity and sensitivity of this new tool will be evaluated in comparison with a previously published conventional PCR test and with isolation followed by morphological identification.

[1] Robin, C., Piou, D., Feau, N., Douzon, G., Schenck, N. and Hansen, E. Root and aerial infections of *Chamaecyparis lawsoniana* by *Phytophthora lateralis*: a new threat for European countries. *Forest Pathology*, in press (2010).

[2] Iloos, R., Andrieux, A., Marçais, B. and Frey, P. Genetic characterization of the hybrid *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Gen. Biol.*, **43** 511-529. (2006).

Poster 61

Differential susceptibility of the commonest Andalusian morphotypes of Holm oak to *Phytophthora cinnamomi*

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The high inter and intrapopulation variability in *Quercus ilex* subsp. *ballota* led to the description of different morphotypes for this oak subspecies. Seedlings of the four main morphotypes of Holm oak present in Andalusia (*macrocarpa*, *expansa*, *microcarpa* and *rotundifolia*) were checked for their susceptibility to *P. cinnamomi* root infection. Seedlings were produced from selected acorns previously characterized as belonging to the four morphotypes and also acorns from a natural hybrid *Q. ilex ballota*-*Q. faginea* were included in artificial inoculation experiments. Plants were infected with water suspensions of *P. cinnamomi* chlamydospores added to the substrate. At the end of the experiments, the infected seedlings showed the aerial symptoms of the root disease: yellowing, wilting and in some cases crown defoliation. Root symptoms consisted in necrosis or absence of feeder



roots. The four morphotypes of Holm oak could be separated in three groups according with foliar symptoms developed: very susceptible (*microcarpa*), susceptible (*expansa*) and moderately susceptible (*rotundifolia* and *macrocarpa*), but there were no great differences in root symptoms, always showing a high degree of necrosis. However, infected hybrids exhibited a low degree of foliar and root symptoms and always significantly lower than infected Holm oak morphotypes. We concluded that *Quercus* species able to hybridize with Holm oak and more tolerant to *P. cinnamomi* root infection (such as *Q. faginea*) should be considered as genitors in future breeding programs against the root disease.

[1] Serrano, M. S., De Vita, P., Carbonero, M. D., Fernández, F., Fernández, P. and Sánchez, M.E. Susceptibility to *Phytophthora cinnamomi* of the commonest morphotypes of Holm oak in southern Spain. *Forest Pathology* doi:10.1111/j.1439-0329.2011.00758.x (2012).

Poster 62

Effectiveness of calcium and potassium fertilizers for control of *Quercus ilex* root rot caused by *Phytophthora cinnamomi*

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Based on the observation that the root rot caused by *P. cinnamomi* on *Q. ilex* has a low incidence and severity in soils with medium-high Ca²⁺ content, the effectiveness of different Ca²⁺ and K⁺ products (CaO, CaCO₃, CaCl₂, Ca(NO₃)₂, CaSO₄, KOH, KNO₃, KCl, KIO₃ and K₂SO₄) on mycelial growth, sporangial and chlamydospore production and sporangial germination (production of zoospores) of *P. cinnamomi* has been tested *in vitro* [1]. Although none of the products inhibited mycelial growth at pH ~ 6, CaO, CaCO₃, CaSO₄, KOH and KIO₃ effectively inhibited the sporangial production of the pathogen and therefore, zoospore production, although none of them were as effective in inhibiting the germination of already formed sporangia. CaO, CaCO₃, K₂SO₄ and CaCl₂ also inhibited the production of chlamydospores. Experiments performed in artificially infested soils treated with the most effective compounds in the *in vitro* experiments (CaO, CaCO₃, CaSO₄, KOH and KIO₃) showed that, in general, Ca²⁺ and K⁺ products induced a decrease in chlamydospore viability greater than registered in non-amended soils. Additionally, greenhouse experiments using the same infested soils showed a significant reduction in the severity of symptoms of Holm oak seedlings planted in amended soils in comparison with seedlings growing in infested but untreated soils [1]. These results suggested that the application of Ca²⁺ amendments (mainly CaO and CaCO₃, but also CaSO₄), and even KOH to the soil in rangelands affected by the pathogen could be an effective tool against the root rot, decreasing the incidence of this serious disease. Now, these products are being tested in field conditions.

[1] Serrano M. S., De Vita, P., Fernández-Rebollo, P. and Sánchez, M. E. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology* **132** 271-279 (2012).



Proteomics analysis of responses to *Phytophthora cinnamomi* in Holm oak

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By using classical ecophysiology and biochemical techniques as well as the more recent –omics ones (proteomics, transcriptomics, and metabolomics) we do pretend to characterize natural variability as well as responses to biotic (*P. cinnamomi*) and abiotic stresses in Holm oak. Our current research and recent publications can be found at the web pages: <http://www.uco.es/investiga/grupos/probiveag/>; <http://www.uco.es/restauracionforestal/>. Data presented at the meeting will be focused on the Holm oak-*P. cinnamomi* interaction.

An overview of research activities on Sudden Oak Death (*Phytophthora ramorum*) at the Canadian Forest Service: results and progress

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Phytophthora ramorum (*Pr*) is an invasive alien pathogen causing a devastating disease known as sudden oak death (SOD) (= ramorum bleeding canker) of oak and tanoak trees in native forests of California and southwestern Oregon (USA). It also causes ramorum leaf blight or ramorum shoot dieback of woody ornamentals, such as rhododendron and camellia, in forests, nurseries, and garden environments. This pathogen can infect more than 120 hosts, several of which being present in Canadian forested and urban areas. The Canadian Food Inspection Agency has detected *Pr* in plants from a few retail garden centers in the Vancouver and Victoria areas of BC. Strict eradication protocols were put into effect to prevent *Pr* from spreading into the surrounding environment. Research activities at the Canadian Forest Service have been mainly carried out to better understand the biology, population genetics, and mitigation measures to help assess the risk associated with *Pr* in Canada. Our presentation will summarize results on: 1) development of DNA markers to identify the *Pr* lineages; 2) efficacy of commercial biocontrol products & fungicides against *Pr* lineages; 3) assessment of the aggressiveness and phenotypic differences among lineages of *Pr* [1]; 4) evaluation of susceptibility of selected tree species common to eastern Canada to infection by *Pr*; [2]; 5) assessment of bioherbicidal efficacy of *Chondrostereum purpureum* registered product “Chontrol®” for control of tanoak and bay laurel resprouts in Oregon and California forests, respectively; and 6) research of putative resistance mechanisms in trees to *Pr*.

[1] Elliott, M., Sumampong, G., Varga, A., Shamoun, S. F., James, D., Masri, S. and Grunwald, N. J. Phenotypic differences among three clonal lineages of *Phytophthora ramorum*. *Forest Pathology*, **41** 7-14 (2011).

[2] Jinek, A., Simard, M., Brière, S. C., Watson, A. K., Tweddell, R. J. and Rioux, D. Foliage susceptibility of six eastern Canadian forest tree species to *Phytophthora ramorum*. *Canadian Journal of Plant Pathology*, **33** 26-37 (2011).



Foliar infection of *Rhododendron* by zoospores and cysts of *Phytophthora pini* Leonian

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Phytophthora pini Leonian, recently re-established from *P. citricola* I [1], is a pathogen with a wide range of forest and nursery hosts. It causes foliar infections in horticultural nurseries in Oregon, where recirculating irrigation systems are common.

Detached leaf assays were conducted to determine the impact of inoculum dose and zoospore agitation on development of foliar infection of *Rhododendron*. Wounded and nonwounded leaves were dipped into suspensions of zoospores that were either untreated, mechanically agitated by vortexing, or pumped through an irrigation sprayer system. Disease severity (lesion area) and incidence (number of lesions per leaf area) were measured over seven days.

At inoculum levels of 10,000 propagules/mL, motile zoospores infected both wounded and nonwounded leaves. Vortexing or pumping resulted in zoospore encystment, and inoculation with these treatments caused disease almost exclusively on wounded leaves. Flow cytometry was used to distinguish propagule type present in motile, vortexed, and pumped inocula. SEM of leaves inoculated with encysted propagules showed germinated cysts with hyphae growing over and around stomata without entering leaf tissue until reaching a wound site.

These findings indicate the importance of zoospore motility in reaching suitable infection sites, and demonstrate the impact of zoospore encystment on disease development. This has implications for disease management in nurseries where pruning wounds are common and the pumping of infested irrigation water may influence zoospore motility.

[1] Hong, C., Gallegly, M. E., Richardson, P. A., Kong, P. *Phytophthora pini* Leonian resurrected to distinct species status. *Mycologia* **103** 351-360 (2011).

Infection of oak (*Quercus robur*) and beech (*Fagus sylvatica*) seedlings growing in elevated CO₂ conditions with pathogenic *Phytophthora* species

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Up to 1 year-old potted oak and beech seedlings growing minimum 6 months in greenhouse chambers under 400 ppm and 800 ppm of CO₂ were infected via soil with *P. quercina* or *P. plurivora* and *P. cactorum*, respectively. Elevated CO₂ concentration facilitated *Phytophthora* infections probably because of stimulating fine roots growth. Contemporary climatic changes facilitate growth and infection by Oomycetes which may cause more damage to the future forest ecosystems.



An ecological role for *Phytophthora* taxon oaksoil in western Oregon riparian ecosystems

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Phytophthora taxon oaksoil, an ITS clade 6 *Phytophthora*, was collected from 58 of 88 transects in western Oregon USA riparian alder ecosystems. From water and rhizosphere sampling between the months of June-October 2010, more than 500 isolates were collected. Continued sampling in 2011-12 revealed consistent high levels of this organism in water. Water samples containing *P. taxon* oaksoil were collected year round with more isolates collected per liter during the summer and fall while leaves were falling and accumulating in waterways. It was found that *P. taxon* oaksoil can sporulate and grow on dried and fresh green alder leaves and petioles floated in water under laboratory conditions. *P. taxon* oaksoil was also easily, repeatedly and frequently isolated from fallen alder leaves but only rarely from necrotic fine roots and never from attached leaves above the waterline. The combined evidence suggests *P. taxon* oaksoil is growing and sporulating from alder leaf debris in riparian ecosystems in western Oregon driving up the number of propagules found in water. Little is known about the roles of *Phytophthora* species in ecosystems beyond the aggressive pathogens, but it is likely that *P. taxon* oaksoil can use plant debris such as leaves as a carbon source and as a substrate for asexual reproduction.

A simple, rapid and inexpensive chemical method for the detection phosphite in plant tissue

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Phosphite (phosphonate) is widely applied to plant communities to control the spread and impact of *Phytophthora* species in natural and peri-urban woodland and forest ecosystems. Determining (1) if phosphite applications have been successfully taken up *in planta*, (2) how phosphite is distributed around plants across seasons, and (3) when plants need to be retreated to maintain effective pathogen control is problematic due to the time and costs associated with current methods. This paper describes a direct chemical method of rapidly and effectively estimating the concentration of phosphite in plant material using a silver nitrate reagent. Glass fiber filter papers (Whatman GF/B) are saturated with acidified silver nitrate (1 M) and dried for 2 hours at 60°C. 20 µL of a PVPP treated aqueous plant extract is then adsorbed on to the filter paper and incubated in the dark at room temperature for 1 hour. The presence of phosphite in the extract reduces the silver ions to elemental silver resulting in a grey-black precipitate that is clearly visible. The method was successfully tested on the roots and leaves of a range of exotic and Australian native plants species from different families and genera which had been treated with 0.3% phosphite. The method is rapid, sensitive and inexpensive, and can detect phosphite at concentrations of 1 mM in 20 µL of aqueous extract from 100 mg of fresh plant material, equivalent to 82 µg g⁻¹ fresh weight, or 20 nmol phosphite per sample. The concentrations detected by the silver nitrate method equated well with the more expensive and less rapid HPLC method that we used to confirm the accuracy of the assay.



Calcium supplementation of soil augments the control of *Phytophthora cinnamomi* by phosphite

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Foliar application of phosphite, a systemic fungicide, to *Phytophthora cinnamomi* infected plants results in the control of disease symptoms and a reduction in the spread and impact of the pathogenic in native plant communities. Calcium ions have also been shown to affect the interaction between *Phytophthora* species and their plant hosts and to reduce the impact and spread of disease caused by soil-borne *Phytophthora* species. Calcium may enhance plant defence mechanisms or interfere with sporangial production, zoospore release and encystment on plant roots. Phosphite has been shown to have similar effects. The addition of calcium salts to soil inhibits the infection of plants by *P. cinnamomi*, and there is a correlation between the incidence in dieback disease caused by *P. cinnamomi* in natural ecosystems and the distribution of calcareous soil. This study used a susceptible Australian native plant species *Banksia leptophylla*, to investigate whether the disease control of *P. cinnamomi* by phosphite could be augmented by soil supplementation with calcium sulphate. The results showed that the effects of applying both calcium and phosphite were synergistic, and that the addition of calcium sulphate to the soil augmented and significantly prolonged the effect of foliar phosphite application. A mechanism involving the disruption of intracellular calcium signatures caused by phosphite induced accumulation of pyrophosphate in the cytosol of *P. cinnamomi* is discussed.

Phytophthora-baiting in Norwegian waterways

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In 2011, a *Phytophthora*-survey was carried out in some selected lakes, rivers and streams in southern Norway. We used rhododendron leaves from the cv. 'Cunningham's White' as baits. Prior to baiting, all leaves were surface sterilized with 70% ethanol and placed in perforated bags (2-3 leaves per bag), each with a styrofoam floater to keep the bait near the surface. The bags were anchored to the shore and left in the water for 6-8 days. All locations were recorded with a field mapping GPS-device. At many locations the leaves had dark and/or water soaked spots when removed from the water. Small sections from the leading edges of the spots were dissected and plated on *Phytophthora*-selective media (PARP or PARPH). We detected six *Phytophthora* spp.; *P. gonapodyides*, *P. lacustris*, *P. plurivora*, *P. pseudosyringae*, *P. ramorum*, and *P. syringae*. *P. plurivora* is known to damage beech (*Fagus sylvatica*) and Norway maple (*Acer platanoides*) on the west coast of Norway, *P. ramorum* has mainly been found outdoors on rhododendron, but is also confirmed on *Pieris japonica*, *Viburnum* spp., American oak (*Quercus* sp.), and bilberries (*Vaccinium myrtillus*) in Norway. Pathogenicity of all six *Phytophthora* spp. will be tested on beech in 2012, because *Phytophthora*-symptoms have been found on beech in the areas where we baited.



***Phytophthora* on trees in Norway**

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During the last decade, a number of *Phytophthora* spp. have been detected from diseased conifer and deciduous trees in landscape plantings, urban forests, and Christmas tree and bough plantations in Norway. We have found *P. megasperma* on subalpine fir (*Abies lasiocarpa*) and linden (*Tilia* sp.), a *P. inundata*-like species on nordmann fir (*A. nordmanniana*), *P. plurivora* on Norway maple (*Acer platanoides*) and beech (*Fagus sylvatica*), *P. ramorum* on oak (*Quercus* sp.), *P. gonapodyides* on grey alder (*Alnus incana*), and *P. cambivora* on noble fir (*A. procera*), subalpine fir, and beech. For some of these findings we have clear indications that this is a result of contaminated, imported plants, a worldwide trend. We find it alarming that some of these pathogens have been detected in urban forests, a first step towards our natural ecosystems.

Epidemiology of *Phytophthora ramorum* and *Phytophthora kernoviae* on *Vaccinium* in the natural environment in the UK.

G. L. Thorp, J. A. Turner, P. Jennings

See Session 3

Subspecies identification of *Phytophthora alni* in alder riparian stands in the Czech Republic

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In the Czech Republic, *Phytophthora alni* was firstly confirmed in 2001. Since the time, the pathogen has been spreading quickly and occupies almost all area of the country. The pathogen attacks *Alnus glutinosa* or *A. incana* to a lesser extent and causes considerable losses of alder trees along hundreds of kilometres of riverbanks [1]. Aim of our work was to perform the identification of the *P. alni* isolates at subspecies level using PCR. The allele-specific PCR primers focused on allele diversity of orthologs of ASF-like, TRP1, RAS-Ypt, and GPA1 genes were selected for the identification [2]. The 88 % of 59 analysed isolates belong to *P. alni* ssp. *alni* while 12 % of that are *P. alni* ssp. *uniformis*.

P. alni ssp. *multiformis* has not been recorded in the country till now. The results follow expectations of more effective spreading of *P. alni* ssp. *alni* based on its higher aggressiveness and ecological advantage compared to remaining two taxa. In comparison, the scattered distribution of *P. alni* ssp. *uniformis* may represent the remains of its former occurrence. Therefore, *P. alni* ssp. *uniformis* may be an indigenous subspecies as hypothesised in literature [2] suppressed by the more aggressive related taxon.

[1] Černý, K. and Strnadová, V. *Phytophthora* alder decline: disease symptoms, causal agent and its distribution in the Czech Republic. *Plant Protect. Sci.*, **46** 12–18 (2010).

[2] Iosif, R., Andrieux, A., Marçais, B. and Frey, P. Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genet Biol.* **43** 511–529 (2006).



The presence of *Phytophthora ramorum* in Greece: The risk of spread into forest ecosystems

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During 2010-2011, in a nationwide survey in Greece, *Phytophthora ramorum* was detected in different areas of the country. It was isolated from symptomatic ornamental plants in nurseries only. Identification of the pathogen was based on morphological and physiological characters as well as DNA sequences. *P. ramorum* was initially detected in central Greece in 2010, on rhododendron plants imported from Belgium. In 2011 the pathogen was found in northern Greece also on rhododendron plants imported from Belgium and in two more localities in southern and central Greece on nursery plants of viburnum and camellia produced in the country.

Weather during late spring and summer is hot and dry in most of the areas where nurseries are located in Greece, thus it is not favourable for the spread of *P. ramorum*; this also facilitates control measures. However, chlamydospores of the pathogen can survive high summer temperatures [2] and can cause new infections during autumn and spring. Greece has a range of climatic variations; there are many suitable habitats for the spread and establishment of the pathogen, including natural oak and beech forests in the highlands as well as maquis scrublands, with evergreen oaks and other hosts of *P. ramorum*. There is also the possibility of susceptible hosts in coniferous forests of Greece. The recent outbreak of the disease in larch plantations of UK [1] shows that the behaviour of *P. ramorum* in new environments is unpredictable and this should make us particularly cautious.

[1] Brasier, C. and Webber, J. Plant Pathology: Sudden larch death. *Nature*, **466** 824–825 (2010).

[2] Fichtner, E.J., Lynch, S.C. and Rizzo, D.M. Detection, Distribution, Sporulation, and Survival of *Phytophthora ramorum* in a California Redwood-Tanoak Forest Soil. *Phytopathology*, **97** 1366-1375 (2007).

AFLP analysis reveals low genetic diversity of *Phytophthora austrocedrae* in Patagonia, Argentina. M. L. Vélez, M. P. A. Coetzee, M. J. Wingfield, M. Rajchenberg, A. G. Greslebin

See Session 1

Phytophthora species from native forests of Patagonia

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The diversity and ecology of *Phytophthora* species in natural ecosystems are little known. In Argentina, particularly in Patagonia, this subject has been poorly studied. The aim of this study was to contribute to the knowledge of *Phytophthora* diversity in Patagonian native forests. Sixteen streams located in mixed forests dominated by *Nothofagus* spp. and *Austrocedrus chilensis* were baited for *Phytophthora* using



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pears and foliage of native trees and shrubs. One hundred and fifty isolates were obtained. About 10 morphospecies were preliminarily discriminated. Species were identified by studying morphological and growth characters as well as ITS region sequences of rDNA. The most frequently isolated species was *P. syringae* (frequency 54%, detected in 12 streams), followed by *P. gonapodyides* (25%, 13 streams) and *P. taxon pgchlamydo* (10%, 6 streams). *P. taxon syrchlamydo* (frequency 1,3%, 2 streams), an unidentified species, clearly differentiated from *P. syringae* by morphology but not by the ITS sequence which differed from the *P. syringae* reference sequence in only 2 bases. Most of other species are in clade 6 and differ morphologically from *P. gonapodyides* and *P. taxon pgchlamydo* but the sequences of ITS regions were insufficient to solve the identity of these isolates which are still under study. Differences in the frequency of isolation of each species from each kind of bait were observed. A review of the *Phytophthora* species recorded in Patagonia together with short descriptions and a discussion about their phylogeny is presented.

Poster 78

Pyrosequencing as a tool for detection of *Phytophthoras*: error rate and risk of false MOTU's

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The most widely used technique to identify *Phytophthoras* in environmental samples relies upon culture-based morphological approaches (selective media and baiting techniques). Beside the high specificity, the low sensitivity coupled to long time required to achieve the results are the main bottlenecks limiting its efficacy. Although several molecular-based approaches can circumvent many of these drawbacks, available molecular detection assays for *Phytophthora* species detect only one or few species. Next-generation sequencing (NGS) technologies offer an opportunity to overcome most of the above-described limitations. In this study pyrosequencing of partial ITS amplicons was used to describe the structure of a DNA mix consisting in 8 *Phytophthora* spp. and *Pythium vexans*. Pyrosequencing resulted in 16 965 reads, specific for specimens present in the artificial sample. A cut-off of 98% is suggested to analyse samples naturally affected by *Phytiaceae*, to absorb sequencing errors and limit the risk of false MOTUs. The pyrosequencing analysis in silico of the ITS region showed PA is a useful molecular tool for the rapid detection of *Phytophthora* spp. However, it cannot provide alone all the information need to understand the ecology of *Phytophthoras* but it can offer an opportunity to increase our understanding of this group of pathogens and their impact on natural and managed vegetation systems.

POSTER 79

Biodiversity of *Phytophthora* community in a costal oak ecosystem in Italy

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Oak decline occurs widely in Italy from north to south of the peninsula in a wide range of environmental conditions. In 2002, Vettraino et al reported the possible association between declined oak stands and the presence of *Phytophthora* species. However so far, no particular attention has been paid to the characterization of *Phytophthoras* community in costal oak ecosystems characterized by seasonal flooding and semi permanent pools of temporary water formed by outcrops of groundwater. In 2012 a survey was conducted in the forest of Palo Laziale (latitude 41° 55' to 41° 56' North and longitude 12° 5'



to 12° 6' West, 7 m asl) in central Italy. The site represents one of the last residual of Tyrrhenian plain forest, which originally covered the coastal areas of Latium, dominated by *Quercus ilex*, *Q. cerris*, *Q. pubescens* and having *Ulmus minor*, *Fraxinus angustifolia*, *F. ornus*, *Sorbus domestica*, *Pinus pinea* as accessory species

A total of 12 *Phytophthora* spp. have been recovered. Based on phylogenetic analysis and morphological and physiological analysis, ten species and one informally designated taxon have been described. Among them some commonly recorded species in oak forests as like as *P. cryptogea*, *P. cinnamomi*, *P. gonapodyides*, *P. megasperma*, *P. plurivora* and some rare and unsuspected species (*P. taxon oaksoil*, *P. psychrophila*, *P. multivora* *P. rosacearum*). The identification of two additional *Phytophthora* species is ongoing.

Vettraino, A.M., Barzanti, G. P., Bianco, M. C. , Ragazzi, A., Capretti, P., Paoletti, E., Luisi, N., Anselmi, N. And A. Vannini. Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. *Forest Pathology*, **32**, 19–28(2002)

POSTER 80

Investigations on control measures for *Phytophthora ramorum* and *Phytophthora kernoviae* in heritage gardens and parks. E. F. Wedgwood, D. Lockley, J. Turner, G. Thorp, B. Henricot

See Session 7

POSTER 81

***Phytophthora obscura* and the widespread bleeding canker of *Aesculus* in Europe**

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In the early nineties of the last century an unusual decline of *Aesculus hippocastanum* could be observed in the southern part of Germany. Old horse chestnut trees in public greens showed symptoms including small and pale green leaves and severe “bleeding cankers” with a cambium necrosis mainly on the stem. The *Phytophthora* isolates baited from soil samples around diseased horse chestnuts were originally thought to be *P. syringae* but shown to belong to the new species *P. obscura* Grünwald & Werres after recent reexamination. They are identical to isolates obtained from *Kalmia latifolia* leaves and from soil underneath *Pieris japonica* in the USA. Phylogenetic analysis revealed that *P. obscura* is genetically closely related to *P. syringae* and *P. austrocedrae*. Together these three taxa define a new subclade 8d of *Phytophthora*. Koch’s postulates could be fulfilled with *Kalmia latifolia*. Controlled infection trials showed that *P. obscura* is pathogenic on *Pieris*, *Rhododendron* and *Aesculus hippocastanum*.

[1] Werres, S., J. Richter, and I. Vesper. Studies on diseased and dead horse chestnuts (*Aesculus hippocastanum* L.) in public green spaces. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes*, **47** 81-85 (1995).

[2] Grünwald N. J., Werres S., Goss E.M., Taylor C.R. and Fieland V.J. *Phytophthora obscura* sp. nov., a new species of the novel *Phytophthora* subclade 8d. Article first published online: (2011) DOI: 10.1111/j.1365-3059.2011.02538.x

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3059.2011.02538.x/abstract>



6th IUFRO Working Party 7.02.09
 “*Phytophthora* in Forests and Natural Ecosystems”
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Variability in response to infection of *Phytophthora cinnamomi* in different families of *Quercus ilex*

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Oak decline, a disease caused by oomycetes of the genus *Phytophthora*, causes substantial yield losses throughout the world, particularly in the southwest of the Iberian Peninsula with the very aggressive species *Phytophthora cinnamomi*. In order to reduce the impact of that pathogen in *Quercus ilex* seedlings, priority is given to genetic control through more resistant progeny. Ten plants of each family were tested. Measure of grown in new roots parameters were used as indicators of *P. cinnamomi* resistance. Resistance levels varied continuously across families from high to low values in all experiments, but family rankings were consistent among experiments. The narrow-sense heritability of the resistance character was high at both families. The resistance of holm oak to *P. cinnamomi* is under moderate genetic control. Selections of lines with high levels of resistance are feasible, and such lines can be used in rehabilitation plantings of holm oak forest sites.

Characterising the distribution of *Phytophthora* taxon Agathis (PTA) in the bark, cambium, and wood of diseased New Zealand kauri (*Agathis australis*).

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Increased numbers of standing dead kauri *Agathis australis* are resulting from the Kauri Dieback disease caused by the root- and collar rot pathogen *Phytophthora* taxon Agathis (PTA) [1]. It is not known whether PTA is likely to be found systemically in kauri, and thus the timber may not be utilised without some knowledge of the risk of spread of the disease to healthy kauri. This knowledge gap has resulted in numerous enquiries to ascertain if dead and dying kauri can be harvested for the trees highly valued timber, for both cultural and commercial use. In order to ascertain the risks associated with the timber being a pathway for PTA spread, a direct plating method has been devised to detect the presence of PTA in the bark, cork cambium, sapwood and heartwood [2]. To augment the direct plating approach, commercial Elisa test kits (e.g. PocketDiagnostic®) and a PTA-specific, Real Time PCR assay will be used to map the presence of PTA in late-stage (chronic-phase) diseased trees. From this empirical data, a set of phytosanitary protocols will be developed to govern the utilisation or secure disposal of diseased kauri trees.

[1] Beever, R.E., Waipara, N.W., Ramsfield, T.D., Dick, M.A. and Horner, I.J. Kauri (*Agathis australis*) under threat from *Phytophthora*? Proceedings 4th IUFRO Working Party 7-02-09 "Phytophthora in Forests and Natural Ecosystems", 26-31st August 2007, Monterey, California, USA (2008).

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***Phytophthora cinnamomi* infection results in changed patterns of fine root production in Scots pine**

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Climate change scenarios for the near future include a higher frequency of extreme events, including floods and storms, drought, and abrupt changes between warm and cold weather conditions, coupled with a gradual increase in mean temperatures and changes in rainfall patterns. These factors will seriously impact host-parasite interactions at the individual tree, forest and landscape levels. *Phytophthora* spp. are virulent pathogens on a wide range of plant species and invasions in previously uncolonized regions have caused major problems. The UK has experienced numerous introductions of invasive alien *Phytophthora* spp. in recent years. Species in the *P. citricola* complex are present, for example. Moreover, *P. cinnamomi* was reported in Scots pine (*Pinus sylvestris*) forests in northern Scotland [1], although its impact in these ecosystems is unclear.

The impacts of *Phytophthora citricola sensu lato* and *P. cinnamomi*, inoculated independently or co-inoculated, on Scots pine under flooding conditions, simulating increased precipitation due to climate change, were examined. Inoculation with *P. citricola* and *P. cinnamomi*, coupled with periodic inundation, led changes in several parameters measured. Root morphology, biomass, and disease severity on the roots changed with time following treatment during interactions with *Phytophthora* spp., although very few plants were killed by these treatments.

[1] Chavarriaga, D., Bodles, W.J.A., Leifert, C., Belbahri, L. & Woodward, S. *Phytophthora cinnamomi* and other fine root pathogens: a threat to north temperate pine forests? *FEMS Microbiol. Letters* **276**:67-74 (2007).

***Phytophthora plurivora* and other Oomycota in an Aberdeenshire watershed**

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Oomycota diversity present in the Waters of Feugh, Aberdeenshire was assessed using baiting with leaves, rice grains and green pepper. Oomycota were recovered from all 12 sites sampled, from the upper, open moorland, down through forested and agricultural zones, to a point near the confluence with the River Dee. Isolates obtained were identified using morphological and molecular methods. *Phytophthora plurivora* was present in a section of the river surrounded by pasture and crop land, with riparian woodland. Numerous *Pythium* species were recovered, mainly *Py. undulatum* and *Py. diclinum*. An unknown *Pythium* species was partially identified. The diversity of Saprolegniales included *Saprolegnia diclina*, *S. hypogyna* and *Achlya colorata*. A possible novel species of *Pythiopsis* and an unrecognized Oomycete were also detected. No direct influence of the surrounding land use on the distribution of these species was determined. Current work is focused on determining changes in Oomycota populations with time in Aberdeenshire watersheds and on the identities of the unknown species.



Quantification of infection and colonization of holm oak (*Quercus ilex*) roots by *Phytophthora cinnamomi* using histological methods

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Phytophthora cinnamomi Rands. is an important pathogen widely distributed in the Northern Hemisphere, with a broad host range. Among others diseases, it is known for its important contribution, together with *Pythium spiculum*, to the rot root, this being considered a main factor involved in the decline of holm oak and cork oak, the most important tree species in the "dehesa" ecosystem of South-Western Spain. The life cycle of these pathogens greatly hampers the study of the interaction within their natural ecosystem, being necessary the development of experiments under controlled conditions in order to unveil the host-pathogen interaction mechanisms. An experiment inoculating *P. cinnamomi* (Pe-90 strain) in *Quercus ilex* L. roots growing on inert substrate was carried out. Semithin root sections of 0.2 µm thickness were obtained and stained with Toluidine Blue-O (TBO). Images were captured and digitally treated to identify the areas corresponding to the different pathogen structures and plant tissues of the sections. Several areal indexes for extracellular, intracellular and survival pathogen structures were obtained and their timeline evolution and spatial development were correlated with the visual observation of the infection and colonization process. *P. cinnamomi* explore external root tissues through the apoplast, reaching parenchymatous and vascular tissues of central cylinder, and using these last tissues for nutrient supply and to extend into new root areas. The studied indexes would be a useful tool for studies focusing on differences between treatments or resistance levels, and the specific responses of the host against the pathogen affecting *P. cinnamomi* development.

New hypothesis on the ploidy of the hybrid species *Phytophthora alni* subsp. *alni*

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Alder decline caused by the *Phytophthora alni* complex is one of the most important diseases in natural ecosystems in Europe in the last 20 years. The emergence of *Phytophthora alni* subsp. *alni* (Paa), the pathogen responsible for the epidemics, is linked to an interspecific hybridization event between two parental species: *Phytophthora alni* subsp. *multiformis* (Pam) and *Phytophthora alni* subsp. *uniformis* (Pau). One of these parental species, *Pau* that has been isolated in several European countries and in North America, specifically in Alaska and Oregon, is exotic to Europe and a diploid species [1] [2]. *Pam* possesses a polyploid genome and should normally be tetraploid [2]. In this study, our aim was to determine the ploidy of the hybrid species Paa by using flow cytometry and Real-Time PCR. Firstly, flow cytometry analysis on suspensions of zoospores allows us to compare the genome size of the three species. Secondly, we designed allele-specific primers and probes in order to quantify the number of copy of



alleles from three single copy nuclear genes by using quantitative PCR. Both results are consistent and indicate that Paa should be a triploid species.

[1] Aguayo, J., Adams, G. C, Halkett, F., Catal, M., Husson, C., Nagy, Z. A., Frey, P. and Marçais, B. Genetic population structure indicates that *Phytophthora alni* subsp. *uniformis* is an invasive species in Europe. Submitted.

[2] Ioos, R., Andrieux, A., Marçais, B. and Frey, P. Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genetics and Biology*, **43** 511-529 (2006).



DISCUSSION SESSION: ADAPTATION AND EVOLUTION

Morphological and physiological adaptability of the genus *Phytophthora*

Thomas Jung

Fitness, selection and evolutionary divergence in *Phytophthora*

Clive Brasier

What is a *Phytophthora* species?

Everett Hansen



Morphological and physiological adaptability of the genus *Phytophthora*

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The genus *Phytophthora* shows a remarkable adaptive flexibility unrivalled by any other oomycete or fungal genus: being outcrossing, inbreeding or sterile; having a partially saprophytic, necrotrophic or biotrophic lifestyle with narrow to very wide host ranges; forming various types of long- and shortterm resting structures; producing sporangia that can be either caducuous or persistent, enabling airborne and/or soilborne spread, and that germinate either directly or indirectly; forming zoospores that following encystment either germinate directly or continue spreading via a secondary zoospore or a microsporangium; and having highly different cardinal temperatures.

Several well-studied forest and aquatic *Phytophthora* species will be used as case studies to demonstrate the fine-tuned and ongoing morphological, physiological and breeding strategy adaptations of *Phytophthoras* to the ecological conditions driving their evolution, and to correct some popular misapprehensions about survival and ecology of *Phytophthora*.

Mainly driven by the prevailing funding policies and the pressure to publish in scientific journals with high impact factors the interests in molecular detection tools and phylogenetic studies on the one hand and morphological, physiological and ecological studies on the other hand have diverged diametrically. Meanwhile, most *Phytophthora* research groups are lacking scientists able to recognise the specific morphological and physiological features of different *Phytophthora* species in order to understand what the phenotype can tell us about their ecology and pathogenicity and get to a more complete picture of the organisms we are dealing with. Not before long the *Phytophthora* community will run out of experts able to teach the required skills to younger scientists with profound negative consequences to *Phytophthora* research in general. This presentation aims to stimulate an intense and possibly quite controversial discussion.



Fitness, selection and evolutionary divergence in *Phytophthora*

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Today we put a lot of effort into demonstrating where *Phytophthora* taxa lie at the ends of the clades in our molecular phylogenetic trees. But to truly understand *Phytophthora* phylogeny we need to evaluate the evolutionary processes that caused related taxa to diverge from each other in the first place: the processes that occurred at the nodes of the trees [1]. Similarly, we need to evaluate the microevolutionary processes that are leading to alterations in genetic structure, to reproductive isolation and perhaps even to new taxa in modern *Phytophthora* populations. Such processes tend to be genetically complex and quantitative and can therefore be more difficult to measure than DNA polymorphisms. Often they fall more within the realm of ecological genetics than population genetics [2]. They can involve studying the *Phytophthora* genetic system: the role of heterozygosity, chromosomal arrangements and patterns of outcrossing and inbreeding. They can involve understanding the components of adaptation and fitness; the balance of sexual versus asexual growth and reproduction; the role of gene flow between the organism's pathogenic and saprotrophic phases; and the role of host specialization [3]. Particularly significant today are the influence of episodic selection events, such as sudden exposure of a *Phytophthora* to crop monoculture or to the nursery environment, or its sudden introduction into a new biogeographic zone. Such events can bring about rapid changes in structure and adaptation in *Phytophthora* populations, including emergence of fitted clones and a potential for rapid genetic modification via interspecific hybridization [4]. These issues will be discussed.

[1] Brasier, C.M. (2009). *Phytophthora* biodiversity: How many *Phytophthora* species are there? In *Phytophthoras in Forests and Natural Ecosystems*. Proc. 4th IUFRO Working Party 7.02.09. General Technical Report PSW-GTR-221. Albany, CA: USDA Forest Service, Pacific Southwest Research Station, pp. 101-115.

[2] Brasier, C.M. (1998). Fitness, continuous variation and selection in fungal populations: an ecological perspective. In *'The structure of fungal populations'* (J. Worrall, ed.) pp. 289-318. Kluwer Academic Publishers, Boston & New York.

[3] Brasier, C.M. (1983). Problems and prospects in *Phytophthora* research. In *'Phytophthora, Its Biology, Ecology and Pathology'* (D.C. Erwin, P.H. Tsao, and S. Bartnicki-Garcia, Eds.) pp. 351-364. American Phytopathological Society, St Paul, Minnesota.

[4] Brasier, C.M. (1995). Episodic selection as a force in fungal microevolution with special reference to clonal speciation and hybrid introgression. *Canadian Journal of Botany* **73**, 1213-1221



What is a *Phytophthora* species?

Everett Hansen

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What is a species? Why do we care? *Phytophthora* species have traditionally been defined by morphology and pathogenicity but we are all familiar with the challenges that morphological descriptions place on identification. The biological species is the operational unit of evolution and any modern discussion of speciation and phylogenetic relationships must be based on this concept. Mayr defined biological species as groups of interbreeding populations reproductively isolated from other such populations. How should such zoologically based ideas be applied to uniparental and asexual organisms? The key features of this definition are its evolutionary orientation and its population basis. *Phytophthora* species can be defined as groups of populations that share a common evolutionary lineage and have maintained genetic similarity in morphology, physiology, and ecological behavior. Such a definition is impractical, however, without tools to measure gene flow. Molecular analytical methods have provided the tools, and increasingly sophisticated population genetics and statistical analysis now allow hypothesis testing. A *Phytophthora* phylogeny based on biological species would seem to be within our grasp. Today's reality, however, is still short of that goal. We now define "phylogenetic species" based solely on DNA sequence similarity and ask how many base pair differences does it take to make a new species? Even when time and resources are available for a thorough population study, we may be limited by gaps in our understanding of speciation processes in populations of clonal or inbreeding organisms and the seeming fluidity of hybridization in some groups.



DISCUSSION SESSION: THE NURSERY PATHWAY

Importance of the nursery pathway for the spread of invasive *Phytophthora* across Europe

Thomas Jung

Patterns of repeated introductions and migrations of *P. ramorum* in North America

Nik Grünwald



Ubiquitous *Phytophthora* infestations of forest, horticultural and ornamental nurseries and plantings demonstrate major failure of plant biosecurity in Europe

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Large-scale *Phytophthora* surveys in (1) forest nurseries, advanced tree nurseries, horticultural nurseries and ornamental nurseries and (2) forest, riparian, amenity, landscape and ornamental plantings and horticultural plantations were conducted by 32 research groups in 21 European countries between 1977 and 2012 with most surveys dating from after 2000. Over all countries and nursery types, 1283 out of 1620 nursery fields and container stands (79.2%) in 563 out of 601 nurseries (93.7%) were found infested by a total of 40 different species and designated taxa of *Phytophthora*. In most nurseries highly deleterious host-*Phytophthora* combinations were found, eg. *Alnus* spp. and *P. alni*; *Quercus* spp. and *P. cambivora*, *P. cinnamomi*, *P. quercina* and/or *P. plurivora*; *Castanea sativa* and *P. cambivora* and/or *P. cinnamomi*; *Fagus sylvatica* and *P. cactorum*, *P. cambivora* and/or *P. plurivora*; *Citrus* spp. and *P. citrophthora* and/or *P. nicotianae*; *Rhododendron* and *P. cinnamomi*, *P. plurivora* and/or *P. ramorum*.

In contrast to many southern European nurseries where wilting and dieback symptoms were quite common, most of the infested plants in intensely managed nurseries in Central and Western Europe with regular applications of several fungicides and fungistatic chemicals appeared visually healthy underpinning the uselessness of international plant health protocols that are primarily based on visual inspections.

In the planting surveys a total of 48 *Phytophthora* taxa were recovered from 1498 of the 2353 tested plantings (63.6%) As with the nursery fields, plants were often infected by the most aggressive pathogens towards the respective host species. Infected plants often showed symptoms such as thinning, chlorosis and dieback of the crown, extensive fine root losses and collar rot.

The average numbers of *Phytophthora* species/taxa per infested nursery and planting were 1.8 and 1.4, respectively. Thirty-two of the *Phytophthora* species/taxa detected are considered exotic invasive species. Amongst them *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. plurivora* and *P. quercina* are widespread in Europe and must be considered as well established in both nurseries/plantations and mature stands. Several *Phytophthora* species/taxa have been found for the first time in Europe, ie. *P. austrocedri*, *P. humicola*, *P. quercetorum*, *P. rosacearum*, *P. taxon citricola*-like V and VI, and *P. taxon gregata*-like; while others have never or only rarely or regionally been recorded from mature stands, ie. *P. kernoviae*, *P. lateralis*, *P. multivora*, *P. pini* and *P. ramorum*. These apparently recent introductions demonstrate that alongside the exponentially increasing volume of imports of living plants from overseas to Europe the unintended introductions of *Phytophthora* species are also increasing dramatically.

According to a conservative calculation 770000 infested forest plantings with a total area of 5.4 million hectares have been established in Europe between 1990 and 2010. Millions of infested landscape plantings and ornamental plantings in the urban-forest interface and tens of thousands of kilometers of roadside and riparian plantings of infested advanced trees and shrubs are completing the dense network of *Phytophthora* infestations across Europe.

The findings of this and previous studies demonstrate major failure of plant biosecurity in Europe which will be discussed.



Patterns of repeated introductions and migration of *P. ramorum* in North America

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P. ramorum has emerged repeatedly in North America and Europe despite concerted efforts to eradicate the pathogen [1]. Here, I explore the repeated emergence of *P. ramorum* in the US reconstructing the pattern and process of emergence since discovery of the pathogen in North America based on research published to date. The NA1 clonal lineage was first introduced into California, most likely on nursery crops [2]. The pathogen subsequently migrated to Oregon, Washington and British Columbia via nursery shipments [3]. Furthermore, several shipments from the West coast also moved the pathogen East to a range of States [3]. The EU1 clonal lineage was moved from Europe into the Pacific Northwest [4]. From its initial introduction to either British Columbia or Washington, this lineage was further distributed to Oregon and California. The NA2 clone was first introduced into British Columbia or Washington and has to date only spread to California. Thus, the EU1 and NA1 clonal lineages remain restricted to the West coast states and provinces, while the NA1 lineage is now found in many states in the East and West. While both mating types have been found in nurseries, to date sexual reproduction has not been detected. It is apparent that the nursery shipments and imports are the cause for repeated introductions and movement of the pathogen across continental North America.

[1] Grünwald, N. J., Garbelotto, M., Goss, E. M., Heungens, K., and Prospero, S. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. *Trends in Microbiology*, 2013:131-138 (2012).

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[3] Goss, E. M., Larsen, M., Chastagner, G. A., Givens, D. R., and Grünwald, N. J. Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. *PLoS Pathog* 5: e1000583 (2009).

[4] Goss, E. M., Larsen, M., Vercauteren, A., Werres, S., Heungens, K. and Grünwald, N. J. *Phytophthora ramorum* in Canada: Evidence for migration within North America and from Europe. *Phytopathology* 101:166-171 (2011).



DISCUSSION SESSION: GEOGRAPHIC ORIGINS OF SPECIES

Searching for Phytophthoras in the forests of Nepal and Taiwan

Andrea Vannini

Variation in *Phytophthora*: a key to historical pathways?

Frans Arentz

Are tropical forests *Phytophthora* hot spots?

Yilmaz Balci



Variation in *Phytophthora*: a key to historical pathways?

Frans Arentz

Consultant Forest Pathologist, Yungaburra, Qld, Australia.

Papua New Guinea (PNG) shares a suite of *Phytophthora* species with Australia and East Asia. It has been postulated that both New Guinea and East Asia lie within a centre of origin for several of these *Phytophthora* spp. [1]. Given that a number of these species are found in forests which have been minimally disturbed until recent times, this raises the question of possible pathways for the movement of *Phytophthora* spp. in the region and the antiquity of these pathways.

In 2011 soil sampling for *Phytophthora* spp. was carried out in forests and agricultural areas in a remote location in PNG. Species isolated included *P. cinnamomi*, *P. cryptogea*, *P. boehmeria*, *P. katsurae* and *P. nicotianae*. There was evidence to suggest that different strains and/or mating types of *P. cinnamomi* were moved between localities on exotic planting stock, supporting published observation that infested nursery stock is a major source of introductions of *Phytophthora* into new geographic areas.

The results of the 2011 survey are reviewed in conjunction with findings of surveys of *Phytophthora* spp. carried out in PNG between 1974 and 1986 [2]. Parameters examined included geographic distribution of the *Phytophthora* spp., variability within each species and host associations. Based on these results, and taking into consideration the likely pattern of early human settlement in the Pacific 4000 years ago, it is postulated that early settlers were instrumental in moving *Phytophthora* spp. together with domesticated food plants as they migrated through the region.

[1] Various authors.

[2] Arentz, F. A key to *Phytophthora* species found in Papua New Guinea with notes on their distribution and morphology. *PNG J. Agric. For. Fish.*, **34** 9-18 (1986).



Are tropical forests *Phytophthora* hot spots?

Yilmaz Balci

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In an effort to discover the species present in tropical rain forests in Latin America two expeditions were conducted. First study site was in lowland Amazon in Iquitos, Peru and the second site at Grenada in West Indies. Sampling consisted of collection of forest soil, necrotic leaf samples recently dropped from the canopy and stream baiting. Samples were identified based on morphological features as well as multilocus sequencing. Species identified from the necrotic foliage from canopy included *P. heveae* and *P. tropicalis* for both sites. However, in Peru one and in Grenada, beside *P. palmivora*, two other new species were isolated. *P. heveae* and *P. tropicalis* were also found from soil samples in Peru. The greatest assemblage of species was found in aquatic environments. *P. tropicalis*, *P. heveae*, *P. palmivora*, *P. macrochlamydospora* and *P. insolita* were identified from streams in Grenada. Additionally, in Peru, three new species and in Grenada four new species were identified. In Grenada, *P. palmivora* and *P. heveae* were isolated from necrotic stem and root tissues of nutmeg (*Myristica fragrans*) trees and data collected suggested that these species are possibly associated with the disease known as nutmeg wilt.



ANNOUNCEMENTS



Forest Phytophthoras, a new international journal and website

Jennifer Parke¹ and Joyce Eberhart²

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Oomycete plant pathogens in the genus *Phytophthora* threaten the biodiversity and sustainability of forest ecosystems worldwide. The new online journal, [Forest Phytophthoras](http://journals.oregondigital.org/ForestPhytophthora/issue/view/261), was created to provide a permanent site for publication of peer-reviewed website articles with digital object identifier (DOI) numbers for archiving and retrieval. The journal provides immediate open access on the principle that making research freely available to the public supports a greater global exchange of knowledge. The journal is available at <http://journals.oregondigital.org/ForestPhytophthora/issue/view/261>.

The website, [Forest Phytophthoras of the World](http://www.ForestPhytophthoras.org) [www.ForestPhytophthoras.org], is an international resource where scientists, students, forest managers, regulators, policy makers and the public can share the latest information on species of *Phytophthora* that affect the world's forests. Management and educational materials for each species are included, often in multiple languages. Other website features include a disease finder, an illustrated glossary, a photo gallery, and a section on *Phytophthora* basics. A searchable reference system with links to scientific publications is available.

The "What's New" column highlights recent publications and news releases pertaining to forest Phytophthoras, and a calendar section announces conferences and other events. Links are provided to a list of *Phytophthora* experts worldwide, archived conference proceedings for the IUFRO Working Party on Phytophthoras in Forest and Natural Ecosystems, proceedings of the Sudden Oak Death Science Symposia, and other *Phytophthora* web resources.

Funding for both projects is provided by the USDA Forest Service PSW Research Station. The journal is hosted by a joint project of Oregon State and University of Oregon Libraries (OJS at OregonDigital.org).



Morphological-Molecular ID Tools of Phytophthora: Lucid & Tabular Keys and Sequencing Analysis

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The genus *Phytophthora* currently contains 121 species with 67 of them having been described during the last 15 years. It is expected that the number will continue to increase, potentially reaching up to 200-600 spp. [1]. Many species are responsible for severe damage to crops and natural ecosystems. Although considerable progress on the taxonomy of the genus has been achieved in the last ten years, accurate identification of species is still challenging in part because of unresolved species complexes [2]. Accurate identification of species is fundamental not only for disease management but also for the implementation of regulatory measures to prevent pathogen spread. Given the rapidly increasing international trade, rapid responses based on accurate pathogen identification are critical for protecting agriculture and natural ecosystems from devastating disease. We have used morphological and molecular data from types and well-authenticated species to build a robust identification resource for all 121 reported species (expandable to cover new species). This resource includes interactive Lucid and Tabular identification keys, images, diagrams, fact sheets, and DNA sequence-based identification platform that is linked with Phytophthora Database (<http://www.phytophthoradb.org>). The Identification Technology Program at the USDA-APHIS-CPHST is consulting with our team to complete this resource. Taxonomic support of *Phytophthora* will be enhanced with the ID tools that we are developing. The ID Tools will be available online and will be updated frequently to cover well-authenticated newly discovered species. We expect the tools will be used by national and international scientists particularly those working in regulatory programs.

[1] Brasier, C. (2009) *Phytophthora* Biodiversity: How many *Phytophthora* species are there?. Proceedings of the Fourth Meeting of IUFRO Working Party S07.02.09. pp. 101-115.

[2] Martin, F.N., Abad, Z.G., Balci, Y., Ivors, K. (2012) Identification and Detection of *Phytophthora*: Reviewing our Progress, Identifying our Needs. *Plant Disease* (Accepted for Publication 2/12).



FIELD TRIPS

Abstracts



6th IUFRO Working Party 7.02.09
“*Phytophthora* in Forests and Natural Ecosystems”
9th-14th September 2012
Córdoba-Spain

Can limestone amendments and pig slurry be considered as control methods for *Quercus ilex* root rot caused by *Phytophthora cinnamomi* in dehesa? Preliminary results from field trials

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The root rot caused by *Phytophthora cinnamomi* is leading to high oak tree mortality in dehesa ecosystems located in southwestern Spain. Experiments performed with artificially infested soil in controlled conditions showed that some Ca²⁺ fertilizers induce a decrease of *P. cinnamomi* infections, mainly due to a significant inhibition of sporangial production [1]. Furthermore, under controlled conditions, pig slurry significantly decreased the inoculum potential in infested soil [2]. The aim of this work has been to evaluate in field conditions the ability of limestone amendments and pig slurry to decrease the infectivity of the pathogen. Experiments with two types of limestone amendments, calcium carbonate (1.500 kg ha⁻¹, OCa richness 55,78%), calcium sulphate (2.500 kg ha⁻¹, OCa richness 32,51%) and pig slurry (3000 l ha⁻¹), were carried out in two oak forests in the province of Huelva (Spain): *Los Bueyes*, an open Holm oak woodland (dehesa), and *Campo Baldío*, a former empty land afforested with Holm and cork oaks 15 years ago. In both farms, trees are infected by *P. cinnamomi*. Soil treatments were applied in autumn of 2010 and 2011. Periodically chemical changes on soil (spring), chlamydospore density and infectivity (spring and autumn), tree defoliation, shoot growth and foliar nutrient content (early summer) were evaluated. Although treatments do not influence the density of chlamydospores in the soil, their ability to cause infections decreased significantly in comparison with control (untreated) plots.

A parallel set of experiments were located in small plots (1 m²) for testing a higher number of fertilizers with 12 replicates (plots) per fertilizer plus controls, distributed in three blocks. In 2010, after soil treatments, 10 Holm oak seedlings per small plot were planted. Seventeen months after plantation, there was an increment in survival in some treated plots in comparison with controls, but some differences were detected among blocks depending on their up or down slope location.

[1] Serrano, M.S., De Vita, P., Fernández, P., Sánchez, M.E. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology* **132** 271-279 (2012).

[2] Vicente, M., Sánchez, M.E., Fernández, P., Trapero, A. Evaluation of biofumigant plants and organic amendments for supresiveness of root rot of *Quercus* spp. caused by *Phytophthora cinnamomi*. *Phytopathology* **99** S134-135 (2009).



Holm oak regeneration in dehesas with presence of the soilborne pathogen *Phytophthora cinnamomi*: something to do?

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Since 1997, near 120 000 ha of former agricultural or abandoned lands have been forested in southern Spain. These afforestation programmes led to a great increase in forest nursery production, but plant quality was not well controlled. *Quercus ilex* spp. *ballota* (Holm oak) and *Q. suber* (cork oak) were the main species planted, occupying 70% of the land afforested in southern Spain in the last 15 years. The quality and health status of oak seedlings produced in the nursery greatly determines the success of afforestations, influencing growth and survival of plants. At the nursery environment, diseases spread easily and quickly and, following infection, symptoms may appear rapidly, but they may also be delayed until the seedlings have been planted out in the forest. *Phytophthora* diseases have rarely been diagnosed in Spanish oak nurseries, and death of seedlings has usually been attributed to a deficient watering or to a bad aeration of the substrates. However, *Phytophthora* species have been demonstrated to be present in Andalusian nurseries [1] and almost certainly they can be considered not only as the main cause of seedling death, but also responsible of initiate infections that result in disease when already infected seedlings were planted out in the forest. The natural soil used as part of planting substrate seems to be the most probable source of inoculum in nurseries. This process is thought to have been an important mean of dissemination of *P. cinnamomi* through the oak forests in southern Spain.

What to do when the pathogen is already established in afforestations? How to manage its quick spread that is killing hundreds of young trees every year?

Different approaches have been addressed at the University of Córdoba: Preventive phosphite treatments [2] or limestone amendments at nursery level [3] [4]. Now, successful treatments are being tested separately or combined at the Holm oak afforestation *Campo Baldío*. Preliminary results will be exposed and discussed.

[1] Sánchez, M.E., Andicoberry, S., Trapero, A. Pathogenicity of three *Phytophthora* spp. causing late seedling rot of *Quercus ilex* ssp. *ballota*. *Forest Pathology* **35** 115-125 (2005).

[2] Navarro, R.M., Terán, A.I., Sánchez, M.E. Acción preventiva y curativa del fosfonato en el control de *Phytophthora cinnamomi* Rands en encina y alcornoque. *Boletín de Sanidad Vegetal Plagas* **32** 685-694 (2006).

[3] Serrano, M.S., De Vita, P., Fernández, P., Sánchez, M.E. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology* **132** 271-279 (2012).

[4] Serrano, M.S., Fernández, P., De Vita, P., Sánchez, M.E. Calcium mineral nutrition increases the tolerance of *Quercus ilex* to *Phytophthora* root disease affecting oak rangelands ecosystems in Spain. *Agroforestry Systems*. In press, doi: 10.1007/s10457-012-9533-5 (2012).



Phytophthora root rot in a wild olive forest (*P. megasperma* and *P. inundata*)

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From the beginning of 90's, wilting and death of cultivated olive trees (*Olea europaea*) are common in southern Spain, mainly affecting new plantations. Death of trees occurs rapidly, with or without previous yellowing or defoliation. In field surveys, most of the affected plantations had waterlogged or very wet soils. In all of these plantations, tree death was associated with root rot. Fungal isolations from affected roots coming from 166 olive orchards all along Andalusia, consistently yielded *Phytophthora* spp. (94% of the orchards showing root rot) [1]. These *Phytophthora* isolates were firstly characterized on the basis of their morphological and physiological properties, and assigned to two distinct groups, A and B, representing 66% and 33% of the isolates respectively. All the group A isolates conformed to *P. megasperma sensu stricto* and its identification was confirmed based on molecular analysis of the ITS regions [2]. Group B isolates exhibited unique characteristics, being heterothallic but with a degree of sporadic self-fertility. All these characteristics and their ITS sequences conformed closely to those of the former 'O-group' taxon described by Brasier et al. In a later study involving O-group isolates from a wide range of hosts, both A1 and A2 mating types were identified and the 'O-group' taxon designated as a new species: *P. inundata* [3].

In the last decade a similar root rot has been detected in afforestations of wild olives and recently also in a mature wild olive woodland (Dehesa de Abajo, Sevilla). In both cases trees were located in seasonal waterlogged soils and *P. megasperma* and *P. inundata* were consistently isolated from necrotic roots and rizosphere. It seems reasonable that susceptibility to *Phytophthora* root rot may have limited the natural ecological distribution of olive trees. It is clear that wild olive should not be planted in conditions where flooding occurs or in areas with poor drainage.

Suitable control measures to decrease infection rates and pathogen dispersal in mature olive forests will be exposed and discussed.

[1] Sánchez, M.E., Ruiz, A., Pérez de Algaba, A., Blanco, M.A., Trapero, A. Occurrence and etiology of death of young olive trees in southern Spain. *European Journal of Plant Pathology* **104** 347-357 (1998).

[2] Sánchez, M.E., Muñoz, M., Brasier, C.M., Trapero, A. Identity and pathogenicity of two *Phytophthora* taxa associated with a new root disease of olive trees. *Plant Disease*, **85** 411-416 (2001).

[3] Brasier, C.M., Sánchez-Hernández, E., Kirk, S.A. *Phytophthora inundata* sp. nov., a part heterothallic pathogen of trees and shrubs in wet or flooded soils. *Mycological Research*, **107** 477-484 (2003).



FIELD TRIPS

Guides



6th IUFRO Working Party 7.02.09
“*Phytophthora* in Forests and Natural Ecosystems”
9th-14th September 2012
Córdoba-Spain

MONDAY, 10th September 2012

MORNING FIELD TRIP: Holm oak (*Quercus ilex*) dehesa

Dehesa *La Jarosa*

Location: Córdoba province

Departure time from Córdoba: 8:30

Estimated arrival time in Córdoba: 13:30



Field Trip Program



Stops:

1. The meaning of dehesa. Management of natural vegetation
2. Multiple uses of Dehesa agroforestry systems
3. Tree management. Cork oak and cork production
4. Livestock and pasture management
5. Control of *Phytophthora* root rot by potassium phosphite applied by trunk injection. Practical display of injection devices commonly used in Spain offered by the technical staff of Fertinyect SL



Dehesa: a traditional Mediterranean agroforest ecosystem

Dehesas are the most widespread agroforestry systems in Europe, covering 3.1 million hectares in the Iberian Peninsula. Dehesas are multipurpose open woodlands, mostly created by clearing natural forests, where livestock rearing, cereal and legume cropping, cork and firewood harvesting and hunting are combined. Most dehesas are characterised by a two-layered vegetation structure: tree layer and an understorey pasture or crop in the same land unit. The tree layer is dominated by the evergreen Holm oak (*Quercus ilex* subsp. *ballota*), cork oak (*Q. suber*) and, to a much lesser extent, by other species. These tree species have a density ranging from 5 to more than 100 trees per hectare but commonly 15–45 trees per hectare in flat areas and up than 50 in the hills. The canopy covers around 20-40% of soil. Holm oak (and cork oak) are regularly thinned and pruned for multiple purposes, such as ensuring maximum yield of acorns and obtaining firewood and charcoal. Most of the pasture species are annual herbs, with a non-vegetative period in summer. In the coldest areas pasture growth also ceases in winter. Pasture yield and quality is also increased through mineral fertilization and sowing of native and introduced species.

Different types of livestock (cattle, sheep, goats, pigs, horses) are common in dehesas. Sheep are the most suitable species; cattle are found in the most humid dehesas, while goats are often used to obtain a better use of woody fodder. Finally, pigs are introduced in the dehesa during October-January for acorn feeding. Livestock are the main tool for maintaining stable understorey vegetation: preventing colonization of pastures by shrubs; improving grass quality and ameliorating soil fertility.

1. Some features about natural environment in dehesa *La Jarosa*

Climate
Climate: Mediterranean Average annual rainfall: 788 mm Average annual temperatures: 15.6°C (24.7° C in August and 7.9° C in January) Maximum temperatures: 40.1° C in August and 18.3° C in January Minimum temperatures: 11.4° C in August and -2.2° C in January Duration of frost period: 6 months (November-April) Duration of dry period: 4 months (June-September)
Topography
The average altitude of the farm is 370 m, ranging between 477 m and 251 m. Topography is hilly. Most farm area has a slope of between 10-20% with flat and rough areas interspersed. South orientation predominates.
Soil
Soils have evolved from slate, quartzite and schists. They are depth and acidic. Type of soils: Lithosol (uphills), Regosol and Cambisol (downhills). Clay: 12% Sand: 54% Silt: 34% pH(1:2.5): 6.5, OM: 2.7%, P available: 9 mg kg ⁻¹ , K available: 177 mg kg ⁻¹ , CEC: 10.5 meq kg ⁻¹ , Ca ²⁺ : 9.2 meq kg ⁻¹ , Mg ²⁺ : 1.3 meq kg ⁻¹
Vegetation
Tree layer is dominated by Holm oak, cork oak and Stone Pine (<i>Pinus pinea</i>). At the shrub layer the most abundant species belong to the genus <i>Cistus</i> (<i>C. crispus</i> , <i>C. ladanifer</i> , <i>C. monspeliensis</i> , <i>C. salvifolius</i> , <i>C. populifolius</i>), but <i>Arbutus unedo</i> , <i>Phillyrea angustifolia</i> , <i>Ruscus aculeatus</i> , <i>Rubia peregrina</i> , <i>Erica australis</i> , <i>Pistacea lentiscus</i> or <i>Pistacea terebinthus</i> are common too.



2. Some features about land use and management of dehesa *La Jarosa*

Farm area
586 ha
Livestock
Cattle: Limousin breed (250 cows; 15 bulls; 25 heifers; 200-220 calves) Swine: Iberian breed. Iberian pig grazes in the farm during fall and early winter. The stocking rate depends on acorn abundance (150-300 pigs)
Forest products
Acorns from Holm oaks and cork oaks (collected by livestock) Cork, debarking every 9 years Pine nuts from Stone Pines
Others uses
Apiculture Pisciculture (Culture of <i>Tinca tinca</i> at the pond) Hunting: red deer (<i>Cervus elaphus hispanicus</i>) and wild boar (<i>Sus scrofa</i>)
Pasture management
Natural pasture: Legumes abundance is the key aspect driving pasture management. Farm is divided in large fences and grazing is conducted in a continuous way but not yearlong. Each fence has a variable rest period. Every 4-5 years, natural pastures are fertilized with 40 kg P ₂ O ₅ ha ⁻¹ . <i>La Jarosa</i> also has an area of irrigated sown pasture, established 30 years ago, being the main species white clover and reed fescue.
Tree management
Holm oak and cork oak were traditionally pruned to provide a standard architecture and to obtain firewood. Now, pruning is limited to special situations like sanitary interventions.
Tree health status
In 2007, one focus of declining Holm oaks was localized. Phytopathological analyses confirmed the presence of <i>P. cinnamomi</i> infecting feeder roots. Symptomatic trees were isolated, avoiding soil movement, and cut when dead. At this moment the oak root disease can be considered as well controlled.



WEDNESDAY, 12th September 2012

DAY TRIP (8:00 TO 19:00) TO ANDALUSIAN MEDITERRANEAN FORESTS

1. Dehesa *Los Bueyes*

Huelva province

Departure time from Córdoba: 8:00

Estimated arrival time: 11:00

DEHESA LOS BUEYES

Dehesa *Los Bueyes* is located at *Las Herrerías*, belonging to the town Hall of *Puebla de Guzmán*, Huelva. This forest is an open woodland mainly composed by mature *Quercus ilex* subsp. *ballota* in an average density of 40 trees per ha. Its topography is hilly with acidic and poor soils. There is a small pond located uphill the area we are visiting.

Since '90s, decline symptoms (defoliation and branch dieback) have been observed affecting trees, leading to high rates of mortality from the 2000's. Soil and roots samples from symptomatic trees were analyzed for the first time in the Laboratory of Agroforest Pathology (University of Córdoba) 12 years ago, leading to the consistent isolation of *Phytophthora cinnamomi*.



Now, several field experiments about control methods to prevent the dissemination of the root disease are conducted at Dehesa *Los Bueyes*, such as soil application of calcium amendments, animal manures or biofumigation. Preliminary results will be exposed and discussed *in situ*.



Orthophoto of *Las Herrerias* in a) 1956, and b) 2007. Yellow area corresponds with the experimental plot

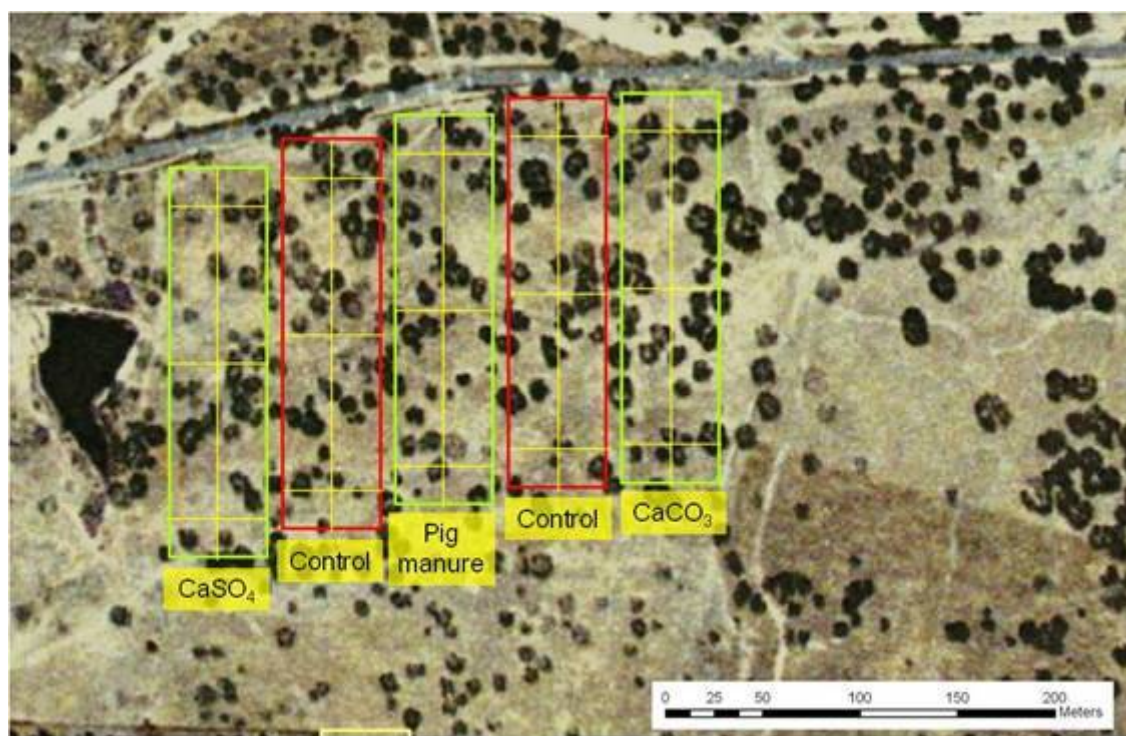
a)



b)



Field experiments at Dehesa *Los Bueyes*. Plots treated with calcium amendments and pig slurry as control methods against *P. cinnamomi* infections



Plot treated with CaCO_3

Small plots treated with different Ca^{2+} amendments



2. *Campo Baldío*

Huelva province

Estimated arrival time: 12:30

CAMPO BALDÍO

Campo Baldío was a communal property of the town Hall of *Puebla de Guzmán* from centuries, used by the inhabitants as pasture grasslands. In 1971, a Civic Company was created by the neighbourhood to improve the exploitation of the property. Olive, citrus and eucalyptus crops were established, but forest lands were also maintained for hunting and Iberian pig breeding.

In the 90's, the unproductive lands at *Campo Baldío* benefited from a state program for afforestation of former agricultural lands. From 1993 to 2008, 850 ha were afforested with Holm and cork oak seedlings planted on terraces at a density of 350-400 plants per ha.

Since 2003, wind turbines and solar energy collectors were installed in *Campo Baldío* for production of green energy.



Oak afforestation at *Campo Baldío*

Almost from the beginning of the afforestation works, foci of dead seedlings were increasingly frequent at *Campo Baldío*. From these initial foci, the root rot caused by *P. cinnamomi* have been spreading, reaching formerly well established young trees that now exhibit defoliation, branch dieback and death.





In 2010, the University of Córdoba chose one plot at Campo Baldío where 10-years old trees are now been infected and killed to set up a second set of field trials to test the effectiveness of calcium amendments, as show in the next Figure. Preliminary results will be exposed and discussed.

Field experiments at *Campo Baldío*. Plots treated with calcium amendments as control methods against *P. cinnamomi* infections



13:30 Lunch at Hostal Andalucía, Villanueva de los Castillejos

15:00 Departure to Dehesa de Abajo



3. Dehesa de Abajo

Sevilla province

Estimated arrival time: 17:00

DEHESA DE ABAJO

Dehesa de Abajo is located at the municipality of *Puebla del Río*, Seville. This forest is mainly composed by the tree species *Pinus pinea* and *Olea europaea* var. *sylvestris*, the last one occupying an area of 17 ha. On the hill there is a pond included at the Andalusian wetland inventory, created through the confinement of the *Majaberraque* watercourse. *Dehesa de Abajo* is cataloged as Natural Reserve and belongs to the Town Hall to *Puebla del Río*.

On March 2009, decline symptoms consisting in severe defoliations and branch dieback were observed in wild olives affecting near 5 ha, being a matter of great concern for the local authorities.



Symptomatic wild olive tree

In *Dehesa de Abajo*, trees affected by decline showed different degree of symptoms depending on their distribution: in the highest places, where the soil is a mixture of gravel and sand, wild olives showed mild to moderate defoliation, while in the lowest sites, where the soil is heavier and wetter, a lot of cases of severe defoliation and death are recorded every year.

This olive forest suffers high fluctuations in water tables due to water extraction for agricultural purposes (the forest is surrounded by rice plantations). In addition, soil humidity always alternate extreme periods of dry and wet conditions.

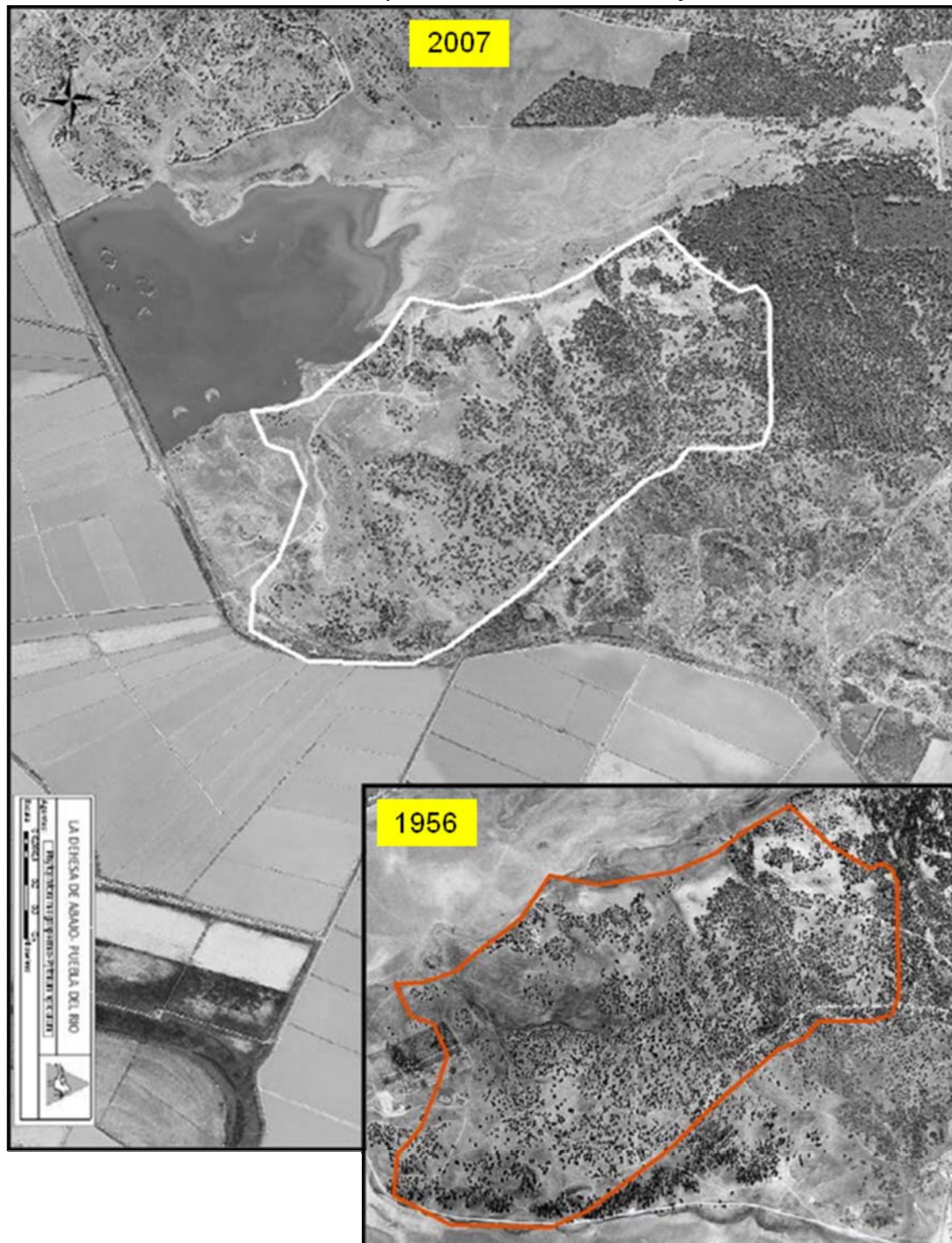
In September 2009, at the beginning of the autumn rainy season, soil and root samples from symptomatic trees were collected by technicians belonging to the Andalusian Government (Agriculture and Environment Council) and sent to the Laboratory of Reference for Andalusia, at



the Agroforest Pathology Unit of the University of Córdoba. In these samples *Phytophthora megasperma* and *P. inundata* were consistently isolated. Analyzing aerial photographs, it is clear that the density of *O. europaea* var. *sylvestris* had been decreasing from down to uphill.

The role of *Phytophthora* species in this forest decline and control measures recommended will be described and discussed.

Evolution of the density of trees in *Dehesa de Abajo* 1956- 2007



18:30 Departure to Córdoba

20:00 Arrival in Córdoba



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“*Phytophthora* in Forests and Natural Ecosystems”
9th-14th September 2012
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